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(71) Applicant (for all designated States except US): **PER-  
FORMANCE PLANTS, INC.** [CA/CA]; BioSciences  
Complex, Kingston, Ontario K7L 3N6 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MCCOURT, Peter**

[CA/CA]; 456 College Street, Toronto, Ontario M6G  
4A3 (CA). **GHASSEMIAN, Majid** [CA/CA]; 1220-1230  
Charles Street West, Toronto, Ontario M4Y 1R5 (CA).  
**CUTLER, Sean** [CA/US]; 460 College Street, Niagra  
Falls, NY 01405 (US). **BONETTA, Dario** [CA/CA];  
1414-1424 Wellesley Steet West, Toronto, Ontario M4Y  
1G1 (CA).

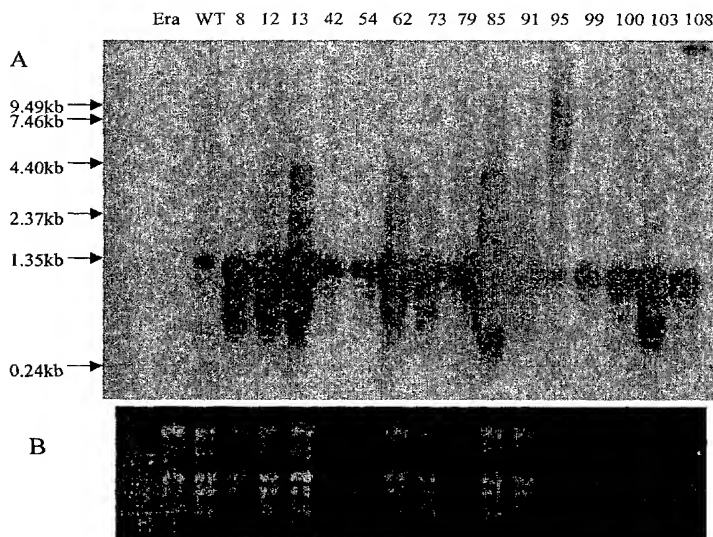
(74) Agent: **ELRIFI, Ivor, R.**; Mintz, Levin, Cohn, Ferris,  
Glovsky, and Popeo, P., C., One Financial Center, Boston,  
MA 02111 (US).

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[Continued on next page]

(54) Title: STRESS TOLERANCE AND DELAYED SENESCENCE IN PLANTS



Northern blot of  $\Delta N90AtFTB$  arabidopsis plants

A. Northern blot probed with  $\Delta N90AtFTB$  DNA probe

B. Ethidium bromide stain of agarose gel showing RNA loading per lane

(57) Abstract: The novel constructs and methods of this invention improve tolerance in plants to environmental stresses and senescence. Nucleic acids encoding a plant farnesyl transferase are described, as are transgenic plants and seeds incorporating these nucleic acids and proteins. Also provided are inhibitors of naturally-occurring farnesyl transferase which, when expressed, will enhance drought tolerance in the plants, improve resistance to senescence and modify growth habit.



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## STRESS TOLERANCE AND DELAYED SENESCENCE IN PLANTS

### BACKGROUND OF THE INVENTION

Most higher plants encounter at least transient decreases in relative water content at some stage of their life cycle and, as a result, have evolved a number of desiccation protection mechanisms. If however, the change in water deficit is prolonged the effects on the plant's growth and development can be profound. Decreased water content due to drought, cold or salt stresses can irreparably damage plant cells which in turn limits plant growth and crop productivity in agriculture.

Plants respond to adverse conditions of drought, salinity and cold with a variety of morphological and physiological changes. Although our understanding of plant tolerance mechanisms to these stresses is fragmentary, the plant hormone abscisic acid (ABA) has been proposed to be an essential mediator between environmental stimulus and plant responses. ABA levels increase in response to water deficits and exogenously applied ABA mimics many of the responses normally induced by water stress. Once ABA is synthesized it causes the closure of the leaf stomata thereby decreasing water loss through transpiration.

The identification of genes that transduce ABA into a cellular response opens the possibility of exploiting these regulators to enhance desiccation tolerance in crop species. In principle, these ABA signalling genes can be coupled with the appropriate controlling elements to allow optimal plant growth and development. Thus, not only would these genes allow the genetic tailoring of crops to withstand transitory environmental insults, they should also broaden the environments where traditional crops can be grown.

In addition, little is known of the genetic mechanisms which control plant growth and development. Genes which further affect other metabolic processes such as senescence and growth habits of plants can be useful in a wide variety of crop and horticultural plants.

### SUMMARY OF THE INVENTION

This invention relates to isolated nucleic acids which encode a farnesyl transferase comprising SEQ ID NO:1 or SEQ ID NO:172. Nucleic acids also encompassed by this invention are such hybridizing sequences which encode the functional equivalent or fragment

thereof of SEQ ID NO:1 or SEQ ID NO:172. The present invention also relates to a method for enhancing the drought tolerance of plants using inhibitors of the products encoded by these nucleic acids. Further, this invention relates to the control of regulatory functions in photosynthetic organisms; for example, in the control of growth habit, flowering, seed production, seed germination, and senescence in such organisms.

This invention also relates to a method for enhancing the drought or stress tolerance of plants by means of alterations in isolated or recombinant nucleic acids encoding a farnesyl transferase (Ftase) protein or fragment thereof or its functional equivalent. Nucleic acids which hybridize to the Ftase-encoding gene (ERA1) are also encompassed by this invention when such hybridizing sequences encode the functional equivalent of the Ftase protein. The present invention also relates to a method for enhancing the drought tolerance of plants through the genetic manipulation of ERA1 gene and its functional equivalents to improve stress tolerance in crop plants. Loss of ERA1 gene function confers enhanced tolerance to drought at the level of the mature plant. The nature of an *era1* mutant with loss of Ftase activity, for example, demonstrates that inhibition of farnesylation enhances ABA responses in a plant.

Further, this invention relates to inhibition of senescence in photosynthetic organisms through inhibition of farnesyl transferase activity. The resulting photosynthetic organisms stay green and tissue viability is maintained for a longer period of time. Thus, methods to provide greener plants and a reduction in senescence are part of this invention.

In yet another embodiment, methods are provided to modify the growth habit and flower induction of plants. Loss of ERA1 gene function under particular environmental conditions results in a reduction in the number of lateral branches produced on a plant and an increase in the number of flowers per inflorescence.

The invention also provides method of producing a transgenic plant, which has an altered phenotype such as increased tolerance to stress (e.g., water deficit, increased biomass, increased yield), delayed senescence or increased ABA sensitivity by introducing into a plant cell a compound that inhibits farnesylation of a polypeptide comprising a CaaX motif. By inhibit Farnesylation is meant to include that the compound inhibits one or more steps in the three step process of farnesylation. In one aspect the compound inhibits farnesyltransferase, prenylprotease or prenylcysteine carboxyl methyltransferase expression or activity. Alternatively, the compound is a anti-sense farnesyl transferase nucleic acid or a farnesyl transferase double stranded RNA-inhibition hair pin nucleic acid. In some aspects the nucleic acid is operably linked to a promoter such as a constitutive promoter, an ABA inducible promoter, tissue specific promoters or a guard cell-specific promoter.



Exemplary anti-antisense nucleic acids are 20 or more consecutive nucleic acids complementary to SEQ ID NO: 1, 14, 40, 43, 80-85 or 172. Alternatively the anti-sense nucleic acids includes SEQ ID NO: 36, 41, 44 or 54-64.

In various aspects the compound is a nucleic acid encoding a farnesyltransferase, prenylprotease or prenylcysteine carboxyl methyltransferase polypeptide of fragment thereof. Alternatively, the compound is a nucleic acid encoding a mutated farnesyltransferase, prenylprotease or prenylcysteine carboxyl methyltransferase polypeptide of fragment thereof. By mutated is meant that the polypeptide lacks at least on activity of the wild type polypeptide such as for example, subunit interaction, substrate binding or enzyme catalysis. A mutated polypeptide forms a dimer, such as a heterodimer. For example, a mutated farnesyltransferase beta polypeptide forms a dimer with a farnesyltransferase alpha polypeptide. Preferably, the polypeptide is less than 400, 350, 314, 300 or 200 amino acids in length. For example, the compound includes SEQ ID NO: 1, 14, 40, 43, 80-85 or 172.

In a further aspect the compound is a nucleic acid encoding a CaaX motif or a nucleic acid encoding a CaaX motif operably linked to a promoter.

Also included in the invention are the plants produced by the methods of the invention and the seed produced by the plants which produce a plant that has an altered phenotype.

This invention also relates to a regulatory sequence useful for genetic engineering of plant cells to provide a method of controlling the tissue pattern of expression of DNA sequences linked to this novel regulatory sequence.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C show the nucleic acid sequence of the ERA1 gene (SEQ ID NO:1) in which the introns are underlined and the start codon (ATG) is at nucleotide positions 1-3.

Figure 2 is the amino acid sequence of the ERA1 protein (SEQ ID NO:2).

Figures 3A-3B show the nucleic acid sequence of the ERA1 promoter (SEQ ID NO:3).

Figure 4 is the amino acid sequence of the  $\beta$  subunit farnesylation domain from *Arabidopsis* (Arab.) (SEQ ID NO:2) aligned with the  $\beta$  subunit farnesylation domains from pea (SEQ ID NO:4), yeast (SEQ ID NO:5) and rat (SEQ ID NO:6). Residues that are identical to the *Arabidopsis* sequence are indicated with a dot. A dash indicates a blank. The amino acid positions of the *Arabidopsis* gene are indicated on the right-hand side.

Figure 5 is a photograph of an *era1*-transformed *Arabidopsis* plant (right) compared to the wild-type (control; *i.e.*, naturally-occurring) plant (left) under extremely dry conditions.

Figure 6 is a graph comparing the water content of *Arabidopsis* plants with inactivated or mutant FtsE activity (*M. Columbia*, *era* 1-2) and controls (M.C. control, *era* 1-2 control).

Figure 7 is a graph comparing the rate of water loss for the *Arabidopsis* plants with inactivated or mutant FtsE activity (*M. Columbia*, *era* 1-2) and controls (M.C. control, *era* 1-2 control).

Figures 8A-8E are comparisons of aging leaves from control (wild-type) and *era*-2 mutant plants.

Figures 9A-9C are comparisons of transcript levels in aging leaves from control (wild-type) and *era*-2 mutant plants.

Figure 10 is an illustration depicting the pBI121 antisense FTA vector construct.

Figure 11 is an illustration of genomic Southern hybridization analysis of anti-FTA transgenic *Arabidopsis thaliana*.

Figure 12 is an illustration of Northern analysis of five 35S-anti-FTA *Arabidopsis thaliana* lines (T3 plants).

Figure 13 shows a Western expression analysis using anti-FTA antibodies to detect the FTA polypeptides.

Figure 14 is a set of photographs showing ABA effects on seedling growth and development. FTA antisense transgenic seedlings exhibit enhanced ABA sensitivity.

Figure 15 shows the effect of ABA on seedling growth and development.

Figure 16 shows photographs of wild type Columbia (A) and four antisense FTA transgenic lines (B, C, D, E) of *Arabidopsis thaliana* after 8 days without watering.

Figure 17 is an illustration of the homology among FTA nucleic acid (A) and amino acid (B) sequences from various plant species based on ClustalW analysis (percent identity shown).

Figure 18 is an illustration of the homology among FTB nucleic acid and amino acid sequences from various plant species based on ClustalW analysis (percent identity shown).

Figure 19 is an illustration of transgenic performance during water stress.

Figure 20 is an illustration of shoot fresh weight, or biomass accumulation, after 6 days of water stress treatment and 6 days recovery time.

Figure 21 is an illustration of seed yield (grams) obtained under optimal conditions or following a 6 day water stress treatment.

Figure 22 is an illustration of vegetative growth under optimal conditions, shown is shoot fresh weight 6 days after the first flower opened.

Figure 23 is an illustration of the effect of a biotic stress coupled with drought stress treatment on seed yield.

Figure 24 is a representative illustration of gel electrophoresis analysis of PCR products in an assay to detect transgenic lines of *Brassica napus*.

Figure 25. is a schematic representation of the vector constructs; A) pBI121-AtCPP, B) pBI121-antisense-AtCPP, C) pBI121-HP-AtCPP.

Figure 26. is an illustration of (A) nucleic acid and (B) amino acid sequence identities as determined by ClustalW analysis.

Figure 27. is a scan of a typical Southern blot of transgenic *Arabidopsis* T1 lines carrying the pBI121-AtCPP construct.

Figure 28. is a scan of a typical Southern blot of transgenic *Arabidopsis* T3 lines carrying the pBI121-HP-AtCPP construct.

Figure 29. is a scan of a typical Southern blot of transgenic *Arabidopsis* lines carrying the pRD29A-AtCPP construct.

Figure 30. is a scan of a typical Southern blot of transgenic *Arabidopsis* lines carrying the pRD29A-HP-AtCPP construct.

Figure 31 is an illustration showing the relative expression of AtCPP mRNA transcript (solid bars) and AtCPP protein levels (stippled bars) in several pBI121-AtCPP transgenic lines.

Figure 32. is a histogram showing the percentage of lines which were categorized as ABA sensitive, moderately ABA sensitive or ABA insensitive. Seedlings were assessed on agar plates containing 1  $\mu$ M ABA and scored at 21 days growth. Thirty-six lines of the pBI121-AtCPP over-expression construct were assessed at 21 days by leaf and seedling development. Thirty-two lines of the 35S-HP-AtCPP down-regulation construct were assessed at 21 days for leaf and seedling development. Each line was assessed by plating approximately 100 seeds per plate and the seedlings scored and recorded as the percent insensitive seedlings per plate. Each line was then expressed as a percent of wild type (Wt). Lines were categorized as sensitive (less than 1% of Wt) solid bars, intermediate (1-50% of Wt) diagonally lined or insensitive (greater than 50% of Wt) stippled, based on their relationship to Wt and the percentage of each category plotted as a histogram.

Figure 33. is an illustration showing the response of wild type and a pRD29A-HP-AtCPP transgenic line to various concentrations of ABA in two week old seedlings.

Figure 34. is a histogram showing the analysis of transgenic plants containing the pBI121-AtCPP over-expression construct, (SEQ ID NO:4). Water loss per gram shoot dry weight after four days of water stress treatment. Lines that are marked with a star are those which were strongly ABA sensitive. Lines marked with a triangle are moderately ABA sensitive. Bars represent means of eight replicates. Lines marked with a filled dot above the bar represents lines which were significantly different from control at a  $p=0.05$  value.

Figure 35. is a histogram showing seed yield in grams of transgenic *Arabidopsis* lines of pBI121-AtCPP grown under optimal water conditions

Figure 36. is a bar chart howing growth and yield of transgenic *Arabidopsis* lines of pBI121-AtCPP grown under optimal watering conditions plus a biotic stress condition. Tields as a % of wild type, rosette leaf number, rosette leaf fresh weight and shoot dry weight are plotted.

Figure 37. are photographs showing rowth of transgenic *Arabidopsis* lines of pBI121-AtCPP grown on agar plates. Changes to root growth visible.

Figure 38. is a bar chart showing rowth of transgenic *Arabidopsis* lines of pRD29A-HP-AtCPP grown under optimal watering conditions. Rosette leaf number, rosette leaf dry weight and shoot dry weight are plotted.

Figure 39 . is an photograph showing Northern blot of  $\Delta N90$ AtFTB arabidopsis plants

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

#### DETAILED DESCRIPTION OF THE INVENTION

This invention relates to transgenic plants that display an altered phenotype, e.g., increased tolerance to stress, delayed senescence, increased ABA sensitivity, increased yield, increased productivity and increased biomass and methods of producing the plants by introducing to a plant cell a compound that inhibits farnesylation of a polypeptide comprising a CaaX motif

Protein farnesylation, the addition of a C-terminal, 15 carbon chain to protein and subsequent processing is a three step enzymatic reaction including farnesylation, proteolytic cleavage and methylation. First, a farnesyltransferase adds the C-terminal 15 carbon chain to a conserved cysteine residue of the CaaX terminal motif, where "C" is a Cystine, "a" is an aliphatic amino acid and "X" is any amino acid. Second, the last three amino acid residues (aaX) are cleaved by a prenyl protease. Lastly, the modified cysteine is methylated by a methylase to create the final active product of the protein farnesylation pathway. The Applicant's have shown that over expression and down-regulation of the alpha or the beta farnesyl transferase gene in plant cells ( i.e, the first step in farnesylation) results in plants with an altered phenotype such as but not limited to drought tolerance and delayed senescence. Applicants have also shown that over expression and down-regulation of the prenyl protease gene (i.e, the second step in farnesylation) in plant cells also results in a plant displaying an altered phenotype including for example but not limited to drought tolerance and increased resistance to biotic and abiotic stress. These results taken together support the hypothesis that modification of the expression of any of the enzymes in the farnesylation pathway (farnesytransferase, prenylprotease or prenylcysteine carboxyl methytransferase in a plant cell will result in a plant displaying an altered phenotype

The present invention also provides novel farnesytransferase (*i.e.*, alpha and beta), (Ftase) and CaaX prenyl protease (CPP) nucleic acid sequences isolated from for example *Arabidopsis thaliana* (At) *Brassica napus* (Bn) and *Glycine Max* (Gm). The invention also provides farnesytransferase and CaaX prenyl protease antisense nucleic acids and constructs comprising these nucleic acids. The sequences are collectively referred to as "PPI nucleic acids", "PPI polynucleotides" or "PPI antisense nucleic acids" and the corresponding encoded polypeptide is referred to as a "PPI polypeptide" or "PPI protein". Unless indicated otherwise, "PPI" is meant to refer to any of the novel sequences disclosed herein. Table A below summarizes the nucleic acids and polypeptides according to the invention

**TABLE A**

PPI Sequence Description	SEQ ID NO:
era1 (FTB)	1
era1 (FTB)	2
ERa1 promoter	3
FTB pea	4
FTB yeast	5
FTB rat	6
At FTA	7

At FTA	8
At FTA	9
pBI121-35S-anti-AtFTA	10
At FTA	11
Bn FTA	12
Bn FTA	13
Bn FTB	14
Bn FTB	15
primer	16
primer	17
primer	18
primer	19
primer	20
primer	21
primer	22
primer	23
primer	24
primer	25
primer	26
primer	27
primer	28
primer	29
primer	30
primer	31
primer	32
primer	33
primer	34
Bn FTA	35
Bn FTB	36
G max FTA	37
G max FTA	38
G max FTA	39
G max FTB	40
G max FTB	41
G max FTB	42
Zea maize FTB	43
Zea maize FTB	44
Zea maize FTB	45
pBI121-35S-AtFTA	46
pBI121-rd29A-anti-AtFTA	47
pBI121-35S-DA-AtFTA	48
pBI121-RD29A-DA-AtFTA	49
MuA-anti-GmFTA	50
RD29A-anti-GmFTA	51

MuA-HP-GmFTA-Nos-Term	52
RD29AP-HP-GmFTA-Nos-Term	53
pBI121-35S-Anti-AtFTB	54
pBI121-RD29AP-Anti-AtFTB	55
pBI121-35S-HP-AtFTB	56
pBI121-RD29AP-HP-AtFTB	57
pBI121-35S-AtFTB	58
MuA-anti-GmFTB-Nos-Term	59
RD29AP-anti-GmFTB-Nos-Term	60
MuA-HP-GmFTB-Nos-Term	61
RD29AP-HP-GmFTB-Nos-Term	62
MuA-anti-Zea maizeFTB-Nos-Term	63
MuA-HP-Zea maizeFTB-Nos-Term	64
Pea-FT-A	65
Tomato-FTA	66
Rice-FT-A	67
Zea mays-FT-A	68
Soy1-Ft-A	69
Soy2-FT-A	70
Triticum-FT-A	71
Pea-FT-A	72
Tomato-FTA	73
Rice-FT-A	74
Zea mays-FT-A	75
Soy1-Ft-A	76
Soy2-FT-A	77
Triticum-FT-A	78
N90AtFTB truncated FTB vector	79
Wiggum (FTB)	80
Dup-Soy-FTB	81
Dup-Corn-FTB	82
Pea-FT-B	83
Tomato-FTB	84

Tobacco-FTB	85
Primer SacI forward	86
Wiggum (FTB)	87
Dup-Soy-FTB	88
Dup-Corn-FTB	89
Pea-FT-B	90
Tomato-FTB	91
Tobacco-FTB	92
Consensus FTA	93
Consensus FTB	94
Consensus FTA	95
Consensus FTB	96
AtCPP	97
AtCPP	98
At-AFC1	
pBI121-AtCPP	99
pBI121-HP-AtCPP	100
AtCPP BamFW	101
AtCPP SmaRV	102
AtCPP-HP-SacFW	103
AtCPP-HP-SacRV	104
pBI121-AtCPP Forward	105
pBI121-antiAtCPP-SmaFW	106
pBI121-antiAtCPP-BamRV	107
p35S-HP-AtCPP Reverse	108
BnCPP	109
BnCPP	110
BnCPP antisense	111
GmCPP	112
GmCPP	113
GmCPP antisense	114
AtCPP antisense	115
BASF-AT1	116
BASF-AT1	117
BASF-AT2	118
BASF-AT2	119
BASF-Corn	120
BASF-Corn	121
BASF-Soy	122
BASF-Soy	123
AFC1	124
AFC1	125
AT4g01320	126
AT4g01320	127
AF007269	128
AF007269	129
pBI121-antisense-AtCPP	130
pRD29A-AtCPP	131
pRD29A-HP-AtCPP	132



pRD29A-antisense-AtCPP	133
MuA-AtCPP	134
MuA-GmCPP	135
pBI121-GmCPP	136
pBI121-HP-GmCPP	137
pBI121-antisense-GmCPP	138
pRD29A-GmCPP	139
pRD29A-HP-GmCPP	140
pRD29A-antisense-GmCPP	141
pBI121-BnCPP	142
pBI121-HP-BnCPP	143
pBI121-antisense-BnCPP	144
pRD29A-BnCPP	145
pRD29A-HP-BnCPP	146
pRD29A-antisense-BnCPP	147
MuA-BnCPP	148
GmCPP SmaFW	149
GmCPP SacRV	150
BnCPP-anti-SmaFW	151
BnCPP-anti-BamRV	152
BnCPP-HP-Sac-FW	153
BnCPP-HP-Sac-RV	154
BnCPP-HP-BamFW	155
BnCPP-HP-XbaRV	156
GmCPP-HP-Sac-FW	157
GmCPP-HP-Sac-RV	158
GmCPP-HP-BamFW	159
GmCPP-HP-XbaRV	160
pRD29AP	161
Nosterm-RV	162
Consensus- BASF	163
Consensus- BASF	164
Consensus- Generic	165
Consensus- Generic	166
Consensus- PPI	167
Consensus- PPI	168
Consensus- PPI/Generic	169
Consensus- PPI/Generic	170
Primer BamHI REV	171
Full Length AtFTB	172
pBI121-AtFTB full length	173
pimer	174
primer	175
isoprenylcysteine carboxyl methyltransferase	176

Full Length AtFTB	177
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This invention also relates to isolated nucleic acids and proteins encoded by these nucleic acids which modify the growth, reproduction and senescence of plants. In particular, the constructs of this invention include an isolated nucleic acid encoding a farnesyl transferase (Ftase) polypeptide comprising SEQ ID NO:1 or 172 or its functional equivalent or fragment thereof, and the Ftase polypeptides or proteins or fragments thereof encoded by these nucleic acids. In particular, this invention relates to a protein wherein the sequence is SEQ ID NO:2 or SEQ ID NO:177.

Further included in this invention are nucleic acid constructs which comprise a promoter (ERA1 promoter) operably-linked to isolated nucleic acid comprising SEQ ID NO:1 or 172 or its functional equivalent or a complement of either. When incorporated into a plant, the ERA1 promoter is regulated in the guard cells of the plant and can affect water loss through the stomates. This promoter consists of a nucleic acid comprising SEQ ID NO:3 (Figure 3).

Transgenic plants, seeds, plant cell and tissues incorporating these constructs are also part of this invention. Accordingly, in one aspect of this invention, a method is provided for producing a gene product under the control of a promoter which operates primarily in guard cells through expression of a gene encoding the gene product in the cell of a plant comprising the steps of: transforming a plant cell with a DNA construct comprising a) a regulatory region comprising SEQ ID NO:3 or a functional portion thereof, DNA comprising a structural gene encoding a gene product, and a 3' untranslated region containing a polyadenylated region; regenerating a plant, photosynthetic organism or tissue culture from the cell; and placing the plant, photosynthetic organisms or tissue culture under conditions so that the promoter induces transcription of the structural gene and the gene product is expressed.

In the context of this disclosure, the terms "regulatory region" or "promoter" refer to a sequence of DNA, usually upstream (5') to the coding sequence of a structural gene, which controls the expression of the coding region by providing recognition and binding sites for RNA polymerase and/or other factors required for transcription to start at the correct site. The term "functional portion" or "functional fragment" refers to a truncated sequence of a promoter of this invention which maintains the capability of inducing transcription of an ERA structural gene under the conditions described for activity of an Ftase protein.

The constructs and methods described herein can be applied to all types of plants and other photosynthetic organisms, including, but not limited to: angiosperms (monocots and dicots), gymnosperms, spore-bearing or vegetatively-reproducing plants and the algae, including the cyanophyta (blue-green algae). Particularly preferred plants are those plants which provide commercially-valuable crops, such as corn, wheat, cotton, rice, canola, sugar cane, sugar beet, sunflowers, potatoes, tomatoes, broccoli, carrots, lettuce, apple, plum, orange, lemon, rose, and the like.

Further, the constructs and methods of this invention can be adapted to any plant part, protoplast, or tissue culture wherein the tissue is derived from a photosynthetic organism. The term "plant part" is meant to include a portion of a plant capable of producing a regenerated plant. Preferable plant parts include roots and shoots and meristematic portions thereof. Other plant parts encompassed by this invention are: leaves, flowers, seeds, epicotyls, hypocotyls, cotyledons, cotyledonary nodes, explants, pollen, ovules, meristematic or embryonic tissue, protoplasts, and the like. Transgenic plants can be regenerated from any of these plant parts, including tissue culture or protoplasts, and also from explants. Methods will vary according to the species of plant.

This invention relates to compositions and constructs comprising isolated nucleic acids (both DNA and RNA) encoding an Ftase and portions thereof of photosynthetic organisms. This invention further relates to compositions and constructs comprising isolated nucleic acids encoding an Ftase promoter. In particular, the ERA1 gene encoding the  $\beta$  subunit of Ftase from *Arabidopsis* and a regulatory sequence which regulates the transcription of the ERA1 gene have been isolated and sequenced. Nucleic acids which encode Ftases from photosynthetic organisms, and homologues or analogs of these nucleic acids, are encompassed by this invention.

The invention further relates to methods using isolated and/or recombinant nucleic acids (DNA or RNA) that are characterized by their ability to hybridize to (a) a nucleic acid encoding an Ftase protein or polypeptide, such as a nucleic acid having the sequences of SEQ ID NO:1 or 172 or (b) a portion of the foregoing (*e.g.*, a portion comprising the minimum nucleotides required to encode a functional Ftase protein; or by the ability to encode a polypeptide having the amino acid sequence of an Ftase (*e.g.*, SEQ ID NO:2 or SEQ ID NO:177, or to encode functional equivalents thereof; *e.g.*, a polypeptide having at least 80% sequence similarity to SEQ ID NO:2 or SEQ ID NO:177, which when incorporated into a plant cell, facilitates the growth habit, seed germination, and metabolism in a photosynthetic organism in the same manner as SEQ ID NO:1 or 172). A functional equivalent of an Ftase therefore, would have at

least an 80% similar amino acid sequence and similar characteristics to, or perform in substantially the same way as, the polypeptide encoded by SEQ ID NO:2 or SEQ ID NO:177 . A nucleic acid which hybridizes to a nucleic acid encoding an Ftase polypeptide such as SEQ ID NO:2 or SEQ ID NO:177 can be double- or single-stranded. Hybridization to DNA such as DNA having the sequence SEQ ID NO:1 or 172, includes hybridization to the strand shown or its complementary strand.

In one embodiment, the percent amino acid sequence similarity between an Ftase polypeptide such as SEQ ID NO:2 or SEQ ID NO:177 , and functional equivalents thereof is at least about 60% ( $\geq 60\%$ ). In a preferred embodiment, the percent amino acid sequence similarity between an Ftase polypeptide and its functional equivalents is at least about 75% ( $\geq 75\%$ ). More preferably, the percent amino acid sequence similarity between an Ftase polypeptide and its functional equivalents is at least about 80%, and still more preferably, at least about 90%, when consecutive amino acids are compared.

Isolated and/or recombinant nucleic acids meeting these criteria comprise nucleic acids having sequences identical to sequences of naturally occurring ERA1 genes and portions thereof, or variants of the naturally occurring genes. Such variants include mutants differing by the addition, deletion or substitution of one or more nucleotides, modified nucleic acids in which one or more nucleotides are modified (e.g., DNA or RNA analogs), and mutants comprising one or more modified nucleotides.

Such nucleic acids, including DNA or RNA, can be detected and isolated by hybridization under high stringency conditions or moderate stringency conditions, for example, which are chosen so as to not permit the hybridization of nucleic acids having non-complementary sequences. "Stringency conditions" for hybridizations is a term of art which refers to the conditions of temperature and buffer concentration which permit hybridization of a particular nucleic acid to another nucleic acid in which the first nucleic acid may be perfectly complementary to the second, or the first and second may share some degree of complementarity which is less than perfect. For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions" and "moderate stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 (see particularly 2.10.8-11) and pages 6.3.1-6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. *et al.*, eds., Vol. 1, containing supplements up through Supplement 29, 1995), the teachings of which are hereby incorporated by reference. The exact conditions which determine the stringency of hybridization depend not only on ionic strength, temperature and the concentration of

destabilizing agents such as formamide, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, high or moderate stringency conditions can be determined empirically.

High stringency hybridization procedures can (1) employ low ionic strength and high temperature for washing, such as 0.015 M NaCl/ 0.0015 M sodium citrate, pH 7.0 (0.1X SSC) with 0.1% sodium dodecyl sulfate (SDS) at 50°C; (2) employ during hybridization 50% (vol/vol) formamide with 5X Denhardt's solution (0.1% weight/volume highly purified bovine serum albumin/ 0.1% wt/vol Ficoll/ 0.1% wt/vol polyvinylpyrrolidone), 50 mM sodium phosphate buffer at pH 6.5 and 5X SSC at 42°C; or (3) employ hybridization with 50% formamide, 5X SSC, 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC and 0.1% SDS. Moderate stringency conditions would be similar except that hybridization would employ 25% formamide in place of 50% formamide.

By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize with the most similar sequences in the sample can be determined.

Exemplary conditions are described in Krause, M.H. and S.A. Aaronson (1991) *Methods in Enzymology*, 200:546-556. Also, see especially page 2.10.11 in *Current Protocols in Molecular Biology* (*supra*), which describes how to determine washing conditions for moderate or low stringency conditions. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids. Generally, from the lowest temperature at which only homologous hybridization occurs, a 1% mismatch between hybridizing nucleic acids results in a 1°C decrease in the melting temperature  $T_m$ , for any chosen SSC concentration. Generally, doubling the concentration of SSC results in an increase in  $T_m$  of  $\approx 17^\circ\text{C}$ . Using these guidelines, the washing temperature can be determined empirically for moderate or low stringency, depending on the level of mismatch sought.

Isolated and/or recombinant nucleic acids that are characterized by their ability to hybridize to (a) a nucleic acid encoding an Ftase polypeptide, such as the nucleic acids depicted as SEQ ID NO:1 or 172, (b) the complement of SEQ ID NO:1 or 172, (c) or a portion of (a) or (b) (*e.g.* under high or moderate stringency conditions), may further encode a protein or polypeptide having at least one functional characteristic of an Ftase polypeptide, such as

regulation of lateral branching under diurnal light cycles, or regulation of the response to ABA, or regulation of senescence.

Enzymatic assays, complementation tests, or other suitable methods can also be used in procedures for the identification and/or isolation of nucleic acids which encode a polypeptide such as a polypeptide of the amino acid sequence SEQ ID NO:2 or SEQ ID NO:177 or a functional equivalent or fragment thereof of this polypeptide. The antigenic properties of proteins or polypeptides encoded by hybridizing nucleic acids can be determined by immunological methods employing antibodies that bind to an Ftase polypeptide such as immunoblot, immunoprecipitation and radioimmunoassay. PCR methodology, including RAGE (Rapid Amplification of Genomic DNA Ends), can also be used to screen for and detect the presence of nucleic acids which encode Ftase-like proteins and polypeptides, and to assist in cloning such nucleic acids from genomic DNA. PCR methods for these purposes can be found in Innis, M.A., *et al.* (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA., incorporated herein by reference.

The nucleic acids described herein are used in the methods of the present invention for production of proteins or polypeptides which are incorporated into cells, tissues, plant parts, plants and other photosynthetic organisms. In one embodiment, DNA containing all or part of the coding sequence for an Ftase polypeptide, or DNA which hybridizes to DNA having the sequence SEQ ID NO:2 or SEQ ID NO:177 is incorporated into a vector for expression of the encoded polypeptide in suitable host cells. The encoded polypeptide consisting of an Ftase subunit or its functional equivalent is capable of farnesyl transferase activity. The term "vector" as used herein refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

Primers and probes consisting of 20 or more contiguous nucleotides of the above-described nucleic acids are also included as part of this invention. Thus, one nucleic acid of this invention comprises a specific sequence of about 20 to about 200 or more nucleotides which are identical or complementary to a specific sequence of nucleotides of the Ftase protein-encoding DNA or transcribed mRNA. These probes and primers can be used to identify and isolate Ftase-encoding nucleic acid from other photosynthetic organisms.

Nucleic acids referred to herein as "isolated" are nucleic acids separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (e.g., as it exists in cells or in a mixture of nucleic acids such as a library), and may have undergone further processing. "Isolated" nucleic acids include nucleic acids obtained by methods described herein, similar methods or other suitable methods, including essentially pure nucleic acids, nucleic acids

produced by chemical synthesis, by combinations of biological and chemical methods, and recombinant nucleic acids which are isolated. Nucleic acids referred to herein as "recombinant" are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures which rely upon a method of artificial recombination, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. "Recombinant" nucleic acids are also those that result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction to the cells of nucleic acids designed to allow or make probable a desired recombination event. Portions of the isolated nucleic acids which code for polypeptides having a certain function can be identified and isolated by, for example, the method of Jasin, M., *et al.*, U.S. Patent No. 4,952,501.

A further embodiment of the invention is antisense nucleic acids or oligonucleotides which are complementary, in whole or in part, to a target molecule comprising a sense strand, and can hybridize with the target molecule. The target can be DNA, or its RNA counterpart (*i.e.*, wherein T residues of the DNA are U residues in the RNA counterpart). When introduced into a cell, antisense nucleic acids or oligonucleotides can inhibit the expression of the gene encoded by the sense strand or the mRNA transcribed from the sense strand. Antisense nucleic acids can be produced by standard techniques. See, for example, Shewmaker, *et al.*, U.S. Patent No. 5,107,065.

In a particular embodiment, an antisense nucleic acid or oligonucleotide is wholly or partially complementary to and can hybridize with a target nucleic acid (either DNA or RNA), wherein the target nucleic acid can hybridize to a nucleic acid having the sequence of the complement of the strand in SEQ ID NO:1 or 172. For example, an antisense nucleic acid or oligonucleotide can be complementary to a target nucleic acid having the sequence shown as the strand of the open reading frame of SEQ ID NO:1 or 172, or nucleic acid encoding a functional equivalent or fragment thereof of Ftase, or to a portion of these nucleic acids sufficient to allow hybridization. A portion, for example, a sequence of 16 nucleotides could be sufficient to inhibit expression of the protein. Fragments comprising 25 or more consecutive nucleotides complementary to SEQ ID NO:1 or 172 could also be used. Or, an antisense nucleic acid or oligonucleotide complementary to 5' or 3' untranslated regions, or overlapping the translation initiation codon (5' untranslated and translated regions), of the ERA1 gene, or a gene encoding a functional equivalent or fragment thereof can also be effective. In another embodiment, the antisense nucleic acid is wholly or partially complementary to and can hybridize with a target nucleic acid which encodes an Ftase polypeptide.

In addition to the antisense nucleic acids of the invention, oligonucleotides can be constructed which will bind to duplex nucleic acid either in the gene or the DNA:RNA complex of transcription, to form a stable triple helix-containing or triplex nucleic acid to inhibit transcription and/or expression of a gene encoding an Ftase polypeptide or its functional equivalent. Frank-Kamenetskii, M.D. and Mirkin, S.M. (1995) *Ann. Rev. Biochem.* 64:65-95. Such oligonucleotides of the invention are constructed using the base-pairing rules of triple helix formation and the nucleotide sequence of the gene or mRNA for Ftase. These oligonucleotides can block Ftase- type activity in a number of ways, including prevention of transcription of the ERA1 gene or by binding to mRNA as it is transcribed by the gene.

Another aspect of the invention pertains to the use of post transcriptional gene silencing (PTGS) to repress gene expression. Double stranded RNA can initiate the sequence specific repression of gene expression in plants and animals. Double stranded RNA is processed to short duplex oligomers of 21-23 nucleotides in length. These small interfering RNA's suppress the expression of endogenous and heterologous genes in a sequence specific manner (Fire et al. *Nature* 391:806-811, Carthew, *Curr. Opin. in Cell Biol.*, 13:244-248, Elbashir et al., *Nature* 411:494-498). A RNAi suppressing construct can be designed in a number of ways, for example, transcription of a inverted repeat which can form a long hair pin molecule, inverted repeats separated by a spacer sequence that could be an unrelated sequence such as GUS or an intron sequence. Transcription of sense and antisense strands by opposing promoters or cotranscription of sense and antisense genes.

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Another aspect of the invention pertains to the use of the dominant-negative genetic approach. Briefly the presence of a dominant trait, i.e. the expression of a transgene, results in a



reduction of enzyme activity or reduced production of the enzymatic end-product. It has been demonstrated that FT is a heterodimer formed by  $\alpha$ - and  $\beta$ - subunits. FT activity relies on the proper dimerization between these subunits to form functional enzyme. Expression of a non-functional subunit will interact with the second subunit to produce a non-functional enzyme and hence reduced enzymatic activity. The non-functional aspect may be in respect to, but not limited to, subunit interaction, substrate binding or enzyme catalysis, for example. Alternatively the expressed trait may produce a substrate analogue which competes with native substrate, the end result being decreased farnesylation of biologically active substrate.

The invention also relates to proteins or polypeptides encoded by the novel nucleic acids described herein. The proteins and polypeptides of this invention can be isolated and/or recombinant. Proteins or polypeptides referred to herein as "isolated" are proteins or polypeptides purified to a state beyond that in which they exist in cells. In a preferred embodiment, they are at least 10% pure; *i.e.*, substantially purified. "Isolated" proteins or polypeptides include proteins or polypeptides obtained by methods described *infra*, similar methods or other suitable methods, and include essentially pure proteins or polypeptides, proteins or polypeptides produced by chemical synthesis or by combinations of biological and chemical methods, and recombinant proteins or polypeptides which are isolated. Proteins or polypeptides referred to herein as "recombinant" are proteins or polypeptides produced by the expression of recombinant nucleic acids.

In a preferred embodiment, the protein or portion thereof has at least one function characteristic of an Ftase; for example, catalytic activity affecting, *e.g.*, normal lateral branching, florets/inflorescence, seed germination, or stomatal opening, and binding function, and/or antigenic function (*e.g.*, binding of antibodies that also bind to naturally occurring Ftase). As such, these proteins are referred to as Ftases of plant origin, and include, for example, naturally occurring Ftase, variants (*e.g.* mutants) of those proteins and/or portions thereof. Such variants include mutants differing by the addition, deletion or substitution of one or more amino acid residues, or modified polypeptides in which one or more residues are modified, and mutants comprising one or more modified residues.

The invention also relates to isolated and/or recombinant portions of an Ftase as described above, especially the  $\beta$  subunit of an Ftase protein. Portions of the enzyme can be made which have full or partial function on their own, or which when mixed together (though fully, partially, or nonfunctional alone), spontaneously assemble with one or more other

polypeptides to reconstitute a functional protein having at least one functional characteristic of an Ftase of this invention.

A number of genes have been identified that are induced by ABA. This suggests that ABA-induced tolerance to adverse environmental conditions is a complex multigenic event. Thus, identification and transfer of single genes into crop plants which improves the viability of the plant under different environmental conditions due to increased responsiveness to ABA is novel and extremely useful.

To identify genes that could be more global controllers of ABA-regulated plant processes, genetic screens were applied in a number of plant species to isolate mutations that alter the response of the plant to the hormone.

Mutations that confer enhanced response to ABA (*era*) in *Arabidopsis* seeds were identified by their ability to prevent seed germination with low concentrations of ABA that normally permit wild-type (controls, *i.e.*, naturally-occurring) seed germination. Of these, the *era1* mutant class, which includes one transferred DNA (T-DNA) line (*era1-1*, ecotype Wassilewskija) and two neutron-generated mutants (*era1-2* and *era1-3*, ecotype Columbia), was of added interest because this class showed decreased germination efficiency under normal postimbibition. Mutations that enhance ABA responsiveness should, in principle, be more dormant. Dormancy in *era1* alleles was alleviated by a 4-day chilling period; the efficiency of *era1* germination increased with the length of time the seeds are chilled. In many plant species, breaking dormancy to allow germination requires vernalization and exposure to moist, low-temperature environments for an extended period (Baskin and Baskin, 1971). The germination profile of *era* mutants could reflect an increased state of ABA-induced dormancy; consequently, these seeds require longer vernalization to germinate. Support for this contention came from construction of double mutants of *era1* with both ABA biosynthetic (*aba1-1*) and insensitive mutants (*abi1-1* and *abi3-6*). In all cases, the double mutants had reduced dormancy as compared with *era 1*, indicating that the increased dormancy observed in *era1* seed was dependent on ABA synthesis or sensitivity.

Aside from broadening the spectrum of new ABA response mutants, supersensitivity screens were also used to identify negative regulators of ABA sensitivity. That is, inhibition of these gene functions enhances the ABA response. One of these genes (ERA1) has been cloned and demonstrated to encode the  $\beta$ -subunit of a heterodimeric protein farnesyl transferase (Ftase) (Cutler *et al.* , 1996). The *era1-1* mutation, which is due to a T-DNA insertion, allowed the isolation of plant genomic regions flanking the insertions. Using the flanking regions as probes, the wild-type cDNA and genomic clones were isolated. Sequence analysis of these described a

gene encompassing 3.5 kb of genomic DNA. The gene contains 13 introns which are underlined in Figures 1A-1C and the T-DNA insertion site in *eral-1* is in intron 8. Southern (DNA) analysis of wild-type DNA, *eral-2*, and *eral-3* probed with *Eral*cDNA revealed that both fast-neutron alleles contain deletions spanning the *ERAL* locus. Fast-neutron mutagenesis induced small deletions in *Arabidopsis* (Shirley *et al.*, 1992), and subsequent genomic analysis with a 14-kb probe that spans the *ERAL* locus determined the size of the *eral-2* deletion to be about 7.5 kb and the *eral-3* deletion to be slightly larger. Thus all three *eral* alleles contained DNA disruptions at the same locus, confirming the identity of the ERA locus.

Conceptual translation of the longest open reading frame (404 amino acids) in the ERA1 gene produced a protein (Figures 2 and 4) with a high sequence similarity to yeast, pea, and mammalian protein farnesyl transferase  $\beta$  subunit genes (Goodman *et al.*, 1988; Chen *et al.*, 1991; Yang *et al.*, 1993). Farnesyl transferases consist of  $\alpha$  and  $\beta$  subunits that dimerize, forming an enzyme that catalyzes the attachment of farnesyl pyrophosphate (15 carbons) to proteins containing a COOH-terminal CaaX motif (Schafer and Rine, 1992), where C designates cysteine residue, aa is usually aliphatic amino acids, and X may designate a cysteine, serine, methionine, or glutamine residue. Both plant  $\beta$  subunit genes contain a region of about 50 amino acids near their COOH-terminus that is absent in yeast and animal  $\beta$  subunit genes.

In yeast and mammalian systems, Ftases modify several signal transduction proteins for membrane localization. This is achieved by the attachment of the lipophilic farnesyl sidechain to the protein target via the Ftase. The attachment of the farnesyl group causes a change in the overall hydrophobicity of the target allowing the protein to anchor itself into the membrane where it usually interacts with other signal transduction molecules. That the loss of farnesylation activity in the *eral* mutant leads to an enhanced response of the seed to ABA suggests a target protein in *Arabidopsis* must be localized to the membrane to attenuate the ABA signal. Thus farnesylation in *Arabidopsis*, appears to be required for the normal function of a negative regulator of ABA sensitivity.

Subsequent work has shown that loss of ERA1 gene function in *Arabidopsis* confers an enhanced tolerance to environmental stresses at the level of the mature plant. For example, a comparison of wild-type plants and *eral* mutant plants grown in soil under standard laboratory conditions (24 hr light, 150  $\mu$ E m<sup>-2</sup>sec<sup>-1</sup>, 30% humidity) showed that the mutants did not require water as frequently as the wild-type plants in order to maintain viability (Figure 5). When mutant and wild-type plants were grown until flowering occurred, watering was stopped

and the plants were observed each subsequent day for signs of stress. Water loss was significantly reduced in the mutant plants compared to the wild-type plants (Figures 6 and 7).

To determine if the observed increased drought tolerance of *era* mutants was related to ERA1 gene function, transgenic plants containing a ERA1 promoter fusion to a reporter GUS gene (made by inserting a 5 Kb fragment of the ERA1 promoter into a promoterless GUS T-DNA plasmid), were constructed. Analysis of the transgenic plants showed that ERA1 is transcriptionally expressed in the epidermal tissue of *Arabidopsis* and that this expression is guard-cell specific. Expression of ERA1 was also noted in the meristematic tissue of the plants and in root hairs. The guard cell expression of ERA1 is consistent with the drought tolerance of the mutant as these cells are the major regulators of water transpiration through the plant. It would be expected that ERA1-regulated stomatal conductance would require expression of the ERA1 gene in the guard cells. Hence loss of ERA1 gene function results in guard cells which are more responsive to ABA which, in turn, leads to more drought responsive guard cell regulation. Therefore, modification of Ftase expression or activity in higher plants, especially crop plants, will have profound effects on stomatal conductance and transpiration rates in the plants.

The nature of the *era1* mutation in *Arabidopsis* demonstrates that inhibition of farnesylation will enhance ABA responses in a plant and alteration of this enzyme activity in crop species. Inhibition of Ftase activity in crop plants can be achieved via a number of methods. For example, antisense technology of cognate ERA1 genes in a variety of crop species can be used to reduce Ftase activity, thus increasing drought tolerance. By specifically producing ERA1 antisense RNA in guard cells, the amount of Ftase synthesized can be reduced to a level which would mimic *era* mutant phenotypes. The ERA1 promoter is regulated in a number of different tissues ranging from shoot meristems to root hairs. By determining the elements of the ERA1 promoter which allow expression in specific tissues, it is possible to tailor the expression of antisense ERA1 to only one tissue or cell type, such as guard cells.

Another method to inhibit Ftase activity in plants is the production of specific peptide inhibitors of farnesylation in transgenic plants. In mammalian and yeast systems, the carboxyl terminal target sequence (CaaX, where C=cysteine, x=aliphatic, X=any amino acid) which allows the attachment of the farnesyl group to specific proteins has been clearly defined. Peptides which mimic these target sequences have been made and shown to inhibit farnesylation of the endogenous target proteins in these systems. Moreover, CAIM is farnesylated *in vivo* in *Arabidopsis*. Thus, similar inhibitors can be applied to higher plants to competitively inhibit Ftase *in vivo*. Again, this can be done through expression of inhibitor peptides in transgenic

plants by synthesizing the DNA sequence for a CaaX peptide and fusing it to a guard cell-specific promoter. In both methods, using the appropriate promoters, antisense Ftsase or peptide inhibitors can be specifically targeted and controlled.

Also included in the invention are methods of producing a transgenic plant. The method includes introducing into one or more plant cells a compound that alters, e.g., inhibits farnesylation of a polypeptide having a carboxyl terminal CaaX motif in the plant to generate a transgenic plant cell and regenerating a transgenic plant from the transgenic cell. In some aspects the compound alters, e.g., increases or decreases CaaX prenyl protease expression or activity. Alternatively, the compound alters farnesyltransferase expression or activity. In other aspects the compound alters isoprenylcysteine carboxyl methyltransferase expression or activity. The compound can be, e.g., (i) a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide; (ii) a nucleic acid encoding a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide; (iii) a nucleic acid that increases expression of a nucleic acid that encodes a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide; (iv) a nucleic acid that decreases the expression of a nucleic acid that encodes a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide; (v) a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase antisense nucleic acid and derivatives, fragments, analogs and homologs thereof. A nucleic acid that increases expression of a nucleic acid that encodes a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide includes, e.g., promoters, enhancers. The nucleic acid can be either endogenous or exogenous. Preferably, the compound is a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide or a nucleic acid encoding a CaaX prenyl protease, farnesyltransferase or isoprenylcysteine carboxyl methyltransferase polypeptide.

Included in the invention are methods of producing a transgenic plant that has increased stress resistance, delayed senescence or increased sensitivity to ABA. The method includes introducing into one or more plant cells a compound that alters farnesyl transferase expression (i.e. farnesyl transferase alpha or beta) or activity in the plant. The compound can be, e.g., (i) a farnesyl transferase polypeptide inhibitor; (ii) a nucleic acid encoding a farnesyl transferase polypeptide inhibitor; (iii) a nucleic acid that decreases expression of a nucleic acid that encodes a farnesyl transferase polypeptide and, derivatives, fragments, analogs and homologs thereof; (iv) an antisense farnesyl transferase nucleic acid. A nucleic acid that decreases expression of a nucleic acid that encodes a farnesyl transferase polypeptide includes, e.g., antisense nucleic

acids or RNA inhibitory nucleic acids. The nucleic acid can be either endogenous or exogenous. Preferably the compound is a farnesyl transferase polypeptide or a nucleic acid encoding a farnesyl transferase polypeptide. More preferably the compound is a nucleic acid complementary to a nucleic acid encoding a farnesyl transferase polypeptide. For example an anti-sense nucleic acid molecule.

Alternatively the compound is a nucleic acid molecule comprising a nucleic acid sequence encoding a mutated farnesyl transferase, isoprenylcysteine carboxyl methyltransferase or CaaX prenyl protease polypeptide. By mutated is meant that the polypeptide lacks one or more function of a wild-type polypeptide. For example, a mutated farnesyltransferase beta polypeptide is a polypeptide has less amino acids than a full length wild type polypeptide by still retains the ability to dimerize with an alpha subunit. For example a mutated farnesyltransferase beta polypeptide is less than 314 amino acids in length. Preferably, the mutated farnesyltransferase beta polypeptide comprises the amino acid sequence of SEQ ID NO:1 or a fragment thereof.

In another aspect the compound is a nucleic acid encoding a CaaX motif. Alternatively, the CaaX motif is operably linked to a promoter.

Also included in the invention is a plant where a mutation has been introduced in the gene encoding farnesyl transferase (i.e. alpha or beta) which results in a plant that has decreased farnesyl transferase activity and increased tolerance to stress as compared to a wild type plant. The mutation may be introduced by chemical or mechanical means.

In various aspects the transgenic plant has an altered phenotype as compared to a wild type plant (*i.e.*, untransformed). By altered phenotype is meant that the plant has a one or more characteristic that is different from the wild type plant. For example, the transgenic plant has an increased resistance to stress. Increased stress resistance is meant that the transgenic plant can grow under stress conditions (*e.g.*, high salt, decreased water, low temperatures, high temperatures) or under conditions that normally inhibit the growth of an untransformed plant. Stresses include, for example, chilling stress, heat stress, heat shock, salt stress, water stress (*i.e.*, drought), nutritional stress, disease, grazing pests, wound healing, pathogens such as for example fungi, bacteria, nematodes, viruses or parasitic weed and herbicides. Methodologies to determine plant growth or response to stress include for example, height measurements, weight or biomass measurements, leaf area or number, ability to flower, water use, transpiration rates and yield. Alternatively, the transformed plant has an increased (*i.e.*, enhanced) ABA sensitivity. The enhanced ABA sensitivity is at the seedling growth stage. Alternatively, the

enhanced ABA sensitivity is at the mature plant stage. Additional altered phenotypes include for example, enhanced vegetative growth (e.g., increased leaf number, thickness and overall biomass), delayed reproductive growth (e.g., flowering later); enhanced seedling vigor (e.g., increased root biomass and length), enhanced lateral root formation and therefore soil penetration more extensive vascular system resulting in an enhanced transport system.

The plant can be any plant type including, for example, species from the genera *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browallia*, *Glycine*, *Pisum*, *Phaseolus*, *Lolium*, *Oryza*, *Zea*, *Avena*, *Hordeum*, *Secale*, *Triticum*, *Sorghum*, *Gossypium*, *Picea*, *Cacao*, and *Populus*.

This invention provides a method of producing drought-tolerant plants comprising: preparing a nucleic acid construct which comprises a promoter operably-linked to a nucleic acid comprising or encoding antisense to SEQ ID NO: 1, 14, 40, 43, 80-85 or 172, or nucleic acid comprising a functional equivalent or fragment thereof of the antisense; inserting the nucleic acid construct into a vector; transforming a plant, tissue culture, or plant cells with the vector; and growing the plant or regenerating a plant from the tissue culture or plant cells; wherein drought-tolerant plants are produced. This method can be used wherein the nucleic acid is selected from the group consisting of 25-200 or more consecutive nucleotides complementary to SEQ ID NO: 1, 14, 40, 43, 80-85 or 172, oligonucleotides consisting of 25 or more consecutive nucleotides of SEQ ID NO: 1, 14, 40, 43, 80-85 or 172 or its complement, or nucleic acid encoding a peptide inhibitor of farnesyl transferase

In addition to stomatal regulation which is extremely sensitive to ABA, *era* plants also demonstrate delayed senescence under drought conditions, indicating that farnesylation negatively regulates a number of drought-induced responses in *Arabidopsis*. The *era* plants grown under normal laboratory conditions take longer to turn yellow. The mutant plants remained green and viable long after the wild-type had senesced and died. Detached leaves of an *era* mutant plant do not yellow as quickly as detached leaves of wild-type plants (Figure 8). Similar-sized leaves which were developmentally identical were taken from wild-type and *era* plants and placed on agar-containing petri plates (See Example 7). Normally, a wild-type leaf begins to lose chlorophyll about five days later and eventually bleaches. The leaves of the mutant

plants remained green for twice as long. Because the leaves were in constant contact with the agar they were not drought stressed, indicating the reduced senescence of the *eral* mutant is not a drought-induced phenomenon.

Moreover, under a 10 hr day/16 hr night cycle, the plant life cycle can be doubled versus the wild-type plants (3 months). It appears therefore, that chlorophyll turnover and senescence signals are altered in the *eral* mutant. For example, wild-type and mutant plants were grown in pots under well-watered conditions to stages of development where the wild-type plant leaves would begin to senesce (about the time of flower development). At this time, developmentally-similar leaves were assayed for senescence-induced marker genes by northern blot analysis (Example 8). Two genes, SAG12 and SAG13, in which transcription is normally induced during senescence in wild-type plants, were not induced in the *eral* mutant (Figure 9). Further, CAB transcription is maintained (Figure 9). Taken together, these results indicate the senescence induction program in *eral* mutants is delayed compared to wild-type plants, showing that loss of farnesylation activity causes a retardation of the induction of senescence in the plant even under conditions wherein water stress is not an environmental factor.

In addition to effects on senescence and water loss, the *eral* mutants show a difference in branching and flowering habit when grown under diurnal light cycles. Under continuous (24 hours light/day) light, the branching pattern of mutants does not differ from that of wild-type plants. However, when given a dark period, the mutants do not produce as many lateral branches as wild-type plants. When measured, plants with loss of farnesylation activity produced only 2.4 branches per plant compared to 3.6 lateral branches per wild-type plant. This represents a 30% decrease in lateral branches per plant.

Flowering is affected by loss of Ftase activity as well. Plants lacking Ftase activity produce more flowers per plant (25-30 buds/inflorescence) than wild-type plants (10-15 buds/inflorescence). Thus, on average there are twice as many flower buds are present on the mutants than on the wild-type plants.

These pleiotrophic effects of the *eral* loss of function mutants on whole plant development indicate that the ERA1 gene can be a coordinate regulator of a collection of plant developmental functions.

Until now, there was no known function for farnesylation in higher plants, including a role in ABA signal transduction. Ftases have been found in a number of higher plants such as tomato and pea, so it is clear that this enzyme has functions across species boundaries. Furthermore, overproduction of farnesyl transferase target peptides or the use of farnesylation inhibitors completely inactivates Ftase in mammalian and yeast systems. Thus, similar



inhibitors can be applied to higher plants to inactivate Ftase *in vivo*. In both cases with the appropriate promoters, antisense Ftase or peptide inhibitors can be specifically targeted and controlled.

The farnesylation deficient mutants are also supersensitive to exogenous auxin. That these mutants show reduced branching and minor alterations in meristem organization, can be explained by altered auxin regulation. Thus other hormone functions are affected in this mutant, which indicates that, in addition to ABA pathways, other hormone regulated pathways are controlled by Ftase activity. These results demonstrate that the ERA1 gene provides a molecular mechanism to coordinate regulation of different hormone signaling molecules.

In accordance with the present invention, the plants included within the scope of this invention are higher and lower plants of the plant kingdom. Mature plants, seedlings and seeds are included in the scope of the invention. A mature plant includes a plant at any stage in development beyond the seedling. A seedling is a very young, immature plant in the early stages of development. Plant parts, protoplasts and tissue culture are also provided by this invention.

Transgenic plants are included within the scope of the present invention which have the phenotype characterized by the *era1* mutation. Seed of transgenic plants are provided by this invention and can be used to propagate more plants containing the constructs of this invention.

ERA1 function in a number of crop plants can be inhibited to enhance the plant's response to adverse environmental conditions that require ABA-mediated signaling. Control of farnesylation in higher plants regulates both embryonic and vegetative tissue response to this hormone (Cutler, *et al.*, 1996). The increased sensitivity translates into a faster response of the tissue to stress conditions which in turn confers increased protection of the plant to the environmental stress. Because this only requires the control of a single gene, ERA1, it should be possible to control farnesylation in a variety of plants by controlling the synthesis or activity of this enzyme. Furthermore, the work described herein clearly indicates that altering the ABA signal transduction pathway by manipulating the genes that control the ABA response makes it possible to improve the plant's response to adverse water stress conditions.

To produce transgenic plants of this invention, a construct comprising the gene encoding Ftase, or nucleic acid encoding its functional equivalent, and a promoter are incorporated into a vector through methods known and used by those of skill in the art. The promoter can comprise all or part of SEQ ID NO:3. The construct can also include any other necessary regulators such as terminators or the like, operably linked to the coding sequence. It can also be beneficial to

include a 5' leader sequence, such as the untranslated leader from the coat protein mRNA of alfalfa mosaic virus (Jobling, S.A. and Gehrke, L. (1987) *Nature* 325:622-625) or the maize chlorotic mottle virus (MCMV) leader (Lommel, S.A., *et al.* (1991) *Virology* 81:382-385). Those of skill in the art will recognize the applicability of other leader sequences for various purposes. Exemplary constructs include SEQ ID NO: 54 -64.

Targeting sequences are also useful and can be incorporated into the constructs of this invention. A targeting sequence is usually translated into a peptide which directs the polypeptide product of the coding nucleic acid sequence to a desired location within the cell, such as to the plastid, and becomes separated from the peptide after transit of the peptide is complete or concurrently with transit. Examples of targeting sequences useful in this invention include, but are not limited to, the yeast mitochondrial presequence (Schmitz, *et al.* (1989) *Plant Cell* 1:783-791), the targeting sequence from the pathogenesis-related gene (PR-1) of tobacco (Cornellisen, *et al.* (1986) *EMBO J.* 5:37-40), vacuole targeting signals (Chrispeels, M.J. and Raikhel, N.V. (1992) *Cell* 68:613-616), secretory pathway sequences such as those of the ER or Golgi (Chrispeels, M.J. (1991) *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42:21-53). Intraorganellar sequences may also be useful for internal sites, *e.g.*, thylakoids in chloroplasts. Theg, S.M. and Scott, S.V. (1993) *Trends in Cell Biol.* 3:186-190.

In addition to 5' leader sequences, terminator sequences are usually incorporated into the construct. In plant constructs, a 3' untranslated region (3' UTR) is generally part of the expression plasmid and contains a polyA termination sequence. The termination region which is employed will generally be one of convenience, since termination regions appear to be relatively interchangeable. The octopine synthase and nopaline synthase termination regions, derived from the Ti-plasmid of *A. tumefaciens*, are suitable for such use in the constructs of this invention.

Any suitable technique can be used to introduce the nucleic acids and constructs of this invention to produce transgenic plants with an altered genome. For grasses such as maize, microprojectile bombardment (see for example, Sanford, J.C., *et al.*, U.S. Patent No. 5,100,792 (1992) can be used. In this embodiment, a nucleotide construct or a vector containing the construct is coated onto small particles which are then introduced into the targeted tissue (cells) via high velocity ballistic penetration. The vector can be any vector which permits the expression of the exogenous DNA in plant cells into which the vector is introduced. The transformed cells are then cultivated under conditions appropriate for the regeneration of plants, resulting in production of transgenic plants.

Transgenic plants carrying the construct are examined for the desired phenotype using a variety of methods including but not limited to an appropriate phenotypic marker, such as antibiotic resistance or herbicide resistance, or visual observation of the time of floral induction compared to naturally-occurring plants.

Other known methods of inserting nucleic acid constructs into plants include Agrobacterium-mediated transformation (see for example Smith, R.H., *et al.*, U.S. Patent No. 5,164,310 (1992)), electroporation (see for example, Calvin, N., U.S. Patent No. 5,098,843 (1992)), introduction using laser beams (see for example, Kasuya, T., *et al.*, U.S. Patent No. 5,013,660 (1991)) or introduction using agents such as polyethylene glycol (see for example Golds, T. *et al.* (1993) *Biotechnology*, 11:95-97), and the like. In general, plant cells may be transformed with a variety of vectors, such as viral, episomal vectors, Ti plasmid vectors and the like, in accordance with well known procedures. The method of introduction of the nucleic acid into the plant cell is not critical to this invention.

The methods of this invention can be used with *in planta* or seed transformation techniques which do not require culture or regeneration. Examples of these techniques are described in Bechtold, N., *et al.* (1993) *CR Acad. Sci. Paris/Life Sciences* 316:118-93; Chang, S.S., *et al.* (1990) *Abstracts of the Fourth International Conference on Arabidopsis Research*, Vienna, p. 28; Feldmann, K.A. and Marks, D.M (1987) *Mol. Gen. Genet.* 208:1-9; Ledoux, L., *et al.* (1985) *Arabidopsis Inf. Serv.* 22:1-11; Feldmann, K.A. (1992) *In: Methods in Arabidopsis Research* (Eds. Koncz, C., Chua, N-H, Schell, J.) pp. 274-289; Chee, *et al.*, U.S. patent, Serial No. 5,376,543.

The transcriptional initiation region may provide for constitutive expression or regulated expression. In addition to the ERA1 promoter, many promoters are available which are functional in plants.

Constitutive promoters for plant gene expression include, but are not limited to, the octopine synthase, nopaline synthase, or mannopine synthase promoters from *Agrobacterium*, the cauliflower mosaic virus (35S) promoter, the figwort mosaic virus (FMV) promoter, and the tobacco mosaic virus (TMV) promoter. Constitutive gene expression in plants can also be provided by the glutamine synthase promoter (Edwards, *et al.* (1990) *PNAS* 87:3459-3463), the maize sucrose synthetase 1 promoter (Yang, *et al.* (1990) *PNAS* 87:4144-4148), the promoter from the Rol-C gene of the TLDNA of Ri plasmid (Sagaya, *et al.* (1989) *Plant Cell Physiol.* 30:649-654), and the phloem-specific region of the pRVC-S-3A promoter (Aoyagi, *et al.* (1988) *Mol. Gen. Genet.* 213:179-185).

Heat-shock promoters, the ribulose-1,6-bisphosphate (RUBP) carboxylase small subunit (ssu) promoter, tissue specific promoters, and the like can be used for regulated expression of plant genes. Developmentally-regulated, stress-induced, wound-induced or pathogen-induced promoters are also useful.

The regulatory region may be responsive to a physical stimulus, such as light, as with the RUBP carboxylase ssu promoter, differentiation signals, or metabolites. The time and level of expression of the sense or antisense orientation can have a definite effect on the phenotype produced. Therefore, the promoters chosen, coupled with the orientation of the exogenous DNA, and site of integration of a vector in the genome, will determine the effect of the introduced gene.

Specific examples of regulated promoters also include, but are not limited to, the low temperature Kin1 and cor6.6 promoters (Wang, *et al.* (1995) *Plant Mol. Biol.* 28:605; Wang, *et al.* (1995) *Plant Mol. Biol.* 28:619-634), the ABA inducible promoter (Marcotte Jr., *et al.* (1989) *Plant Cell* 1:969-976), heat shock promoters, such as the inducible hsp70 heat shock promoter of *Drosophila melanogaster* (Freeling, M., *et al.* (1985) *Ann. Rev. of Genetics* 19: 297-323), the cold inducible promoter from *B. napus* (White, T.C., *et al.* (1994) *Plant Physiol.* 106:917), the alcohol dehydrogenase promoter which is induced by ethanol (Nagao, R.T., *et al.*, Mifflin, B.J., Ed. *Oxford Surveys of Plant Molecular and Cell Biology*, Vol. 3, p 384-438, Oxford University Press, Oxford 1986), the phloem-specific sucrose synthase ASUS1 promoter from *Arabidopsis* (Martin, *et al.* (1993) *Plant J.* 4:367-377), the ACS1 promoter (Rodrigues-Pousada, *et al.* (1993) *Plant Cell* 5:897-911), the 22 kDa zein protein promoter from maize (Unger, *et al.* (1993) *Plant Cell* 5:831-841), the ps1 lectin promoter of pea (de Pater, *et al.* (1993) *Plant Cell* 5:877-886), the phas promoter from *Phaseolus vulgaris* (Frisch, *et al.* (1995) *Plant J.* 7:503-512), the lea promoter (Thomas, T.L. (1993) *Plant Cell* 5:1401-1410), the E8 gene promoter from tomato (Cordes, *et al.* (1989) *Plant Cell* 1:1025-1034), the PCNA promoter (Kosugi, *et al.* (1995) *Plant J.* 7:877-886), the NTP303 promoter (Weterings, *et al.* (1995) *Plant J.* 8:55-63), the OSEM promoter (Hattori, *et al.* (1995) *Plant J.* 7:913-925), the ADP GP promoter from potato (Muller-Rober, *et al.* (1994) *Plant Cell* 6:601-604), the Myb promoter from barley (Wissenbach, *et al.* (1993) *Plant J.* 4:411-422), and the plastocyanin promoter from *Arabidopsis* (Vorst, *et al.* (1993) *Plant J.* 4:933-945).

The vector can be introduced into cells by a method appropriate to the type of host cells (*e.g.*, transformation, electroporation, transfection). For the purposes of this disclosure, the terms "transformed with", "transformant", "transformation", "transfect with", and "transfection" all refer to the introduction of a nucleic acid into a cell by one of the numerous methods known

to persons skilled in the art. Transformation of prokaryotic cells, for example, is commonly achieved by treating the cells with calcium chloride so as to render them "competent" to take up exogenous DNA, and then mixing such DNA with the competent cells. Prokaryotic cells can also be infected with a recombinant bacteriophage vector.

Nucleic acids can be introduced into cells of higher organisms by viral infection, bacteria-mediated transfer (*e.g.*, *Agrobacterium* T-DNA delivery system), electroporation, calcium phosphate co-precipitation, microinjection, lipofection, bombardment with nucleic-acid coated particles or other techniques, depending on the particular cell type. For grasses such as corn and sorghum, microprojectile bombardment as described, for example, in Sanford, J.C., *et al.*, U.S. Patent No. 5,100,792 (1992) can be used. Other useful protocols for the transformation of plant cells are provided in Gelvin *et al.*, 1992. Suitable protocols for transforming and transfecting cells are also found in Sambrook *et al.*, 1989. The nucleic acid constructs of this invention can also be incorporated into specific plant parts such as those described *supra* through the transformation and transfection techniques described herein.

To aid in identification of transformed plant cells, the constructs of this invention are further manipulated to include genes coding for plant selectable markers. Useful selectable markers include enzymes which provide for resistance to an antibiotic such as gentamycin, hygromycin, kanamycin, or the like. Similarly, enzymes providing for production of a compound identifiable by color change such as GUS ( $\beta$ - glucuronidase), or by luminescence, such as luciferase, are useful.

For example, antisense Ftase can be produced by integrating a complement of the ERA1 gene linked to DNA comprising SEQ ID NO:3 into the genome of a virus that enters the host cells. By infection of the host cells, the components of a system which permits the transcription of the antisense present in the host cells.

When cells or protoplasts containing the antisense gene driven by a promoter of the present invention are obtained, the cells or protoplasts are regenerated into whole plants. The transformed cells are then cultivated under conditions appropriate for the regeneration of plants, resulting in production of transgenic plants. Choice of methodology for the regeneration step is not critical, with suitable protocols being available for many varieties of plants, tissues and other photosynthetic organisms. See, *e.g.*, Gelvin S.B. and Schilperoort R.A., eds. *Plant Molecular Biology Manual, Second Edition*, Suppl. 1 (1995) Kluwer Academic Publishers, Boston MA, U.S.A.

Transgenic plants carrying the construct are examined for the desired phenotype using a variety of methods including but not limited to an appropriate phenotypic marker, such as

antibiotic resistance or herbicide resistance as described *supra*, or visual observation of their growth compared to the growth of the naturally-occurring plants under the same conditions.

As used herein, the term transgenic plants includes plants that contain either DNA or RNA which does not naturally occur in the wild type (native) plant or known variants, or additional or inverted copies of the naturally-occurring DNA and which is introduced as described herein. Transgenic plants include those into which isolated nucleic acids have been introduced and their descendants, produced from seed, vegetative propagation, cell, tissue or protoplast culture, or the like wherein such alteration is maintained.

Such transgenic plants include, in one embodiment, transgenic plants which are angiosperms, both monocotyledons and dicotyledons. Transgenic plants include those into which DNA has been introduced and their progeny, produced from seed, vegetative propagation, cell, tissue or protoplast culture, or the like.

Seed can be obtained from the regenerated plant or from a cross between the regenerated plant and a suitable plant of the same species. Alternatively, the plant can be vegetatively propagated by culturing plant parts under conditions suitable for the regeneration of such plant parts.

In yet another aspect of this invention are provided plant tissue culture and protoplasts which contain DNA comprising antisense or an altered ERA1 nucleic acid operably linked to an ERA1 promoter, which alters the response of the tissue culture or protoplasts to varying environmental conditions.

The methods of this invention can also be used with *in planta* or seed transformation techniques which do not require culture or regeneration. Examples of these techniques are described in Bechtold, N., *et al.* (1993) *CR Acad. Sci. Paris/Life Sciences* 316:118-93; Chang, S.S., *et al.* (1990) *Abstracts of the Fourth International Conference on Arabidopsis Research*, Vienna, p. 28; Feldmann, K.A. and Marks, D.M (1987) *Mol. Gen. Genet.* 208:1-9; Ledoux, L., *et al.* (1985) *Arabidopsis Inf. Serv.* 22:1-11; Feldmann, K.A. (1992) *In: Methods in Arabidopsis Research* (Eds. Koncz, C., Chua, N-H, Schell, J.) pp. 274-289; Chee, *et al.*, U.S. patent, Serial No. 5,376,543.

The isolated nucleic acid molecules of the invention can be used to express PPI protein (e.g., via a recombinant expression vector in a host cell), to detect PPI mRNA (e.g., in a biological sample) or a genetic lesion in a PPI gene, and to modulate PPI activity, as described further, below. In addition, the PPI proteins can be used to screen compounds that modulate the PPI protein activity or expression. In addition, the anti-PPI antibodies of the invention can be used to detect and isolate PPI proteins and modulate PPI activity.

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to PPI proteins or have a stimulatory or inhibitory effect on, *e.g.*, PPI protein expression or PPI protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to a PPI protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.*, Lam, 1997. *Anticancer Drug Design* 12: 145. A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678; Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor, 1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a PPI protein, or a biologically-active portion thereof, is contacted with a test compound and the ability of the test compound to bind to a PPI protein determined. The cell, for example, can be of mammalian origin, plant cell or a yeast cell. Determining the ability of the test compound to bind to the PPI protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the PPI protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a PPI protein, or a biologically-active portion thereof, with a known compound which binds PPI to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PPI protein, wherein determining the ability of the test compound to interact with a PPI protein comprises determining the ability of the test compound to preferentially bind to PPI protein or a biologically-active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a PPI protein, or a biologically-active portion thereof, with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the PPI protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of PPI or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the PPI protein to bind to or interact with a PPI target molecule. As used herein, a "target molecule" is a molecule with which a PPI protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a PPI interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A PPI target molecule can be a non-PPI molecule or a PPI protein or polypeptide of the invention. In one embodiment, a PPI target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.* a signal generated by binding of a compound to a membrane-bound molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that



has catalytic activity or a protein that facilitates the association of downstream signaling molecules with PPI.

Determining the ability of the PPI protein to bind to or interact with a PPI target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the PPI protein to bind to or interact with a PPI target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular  $\text{Ca}^{2+}$ , diacylglycerol,  $\text{IP}_3$ , etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a PPI-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising contacting a PPI protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the PPI protein or biologically-active portion thereof. Binding of the test compound to the PPI protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the PPI protein or biologically-active portion thereof with a known compound which binds PPI to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PPI protein, wherein determining the ability of the test compound to interact with a PPI protein comprises determining the ability of the test compound to preferentially bind to PPI or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting PPI protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity of the PPI protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of PPI can be accomplished, for example, by determining the ability of the PPI protein to bind to a PPI target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of PPI protein can be accomplished by determining the ability of the PPI protein further modulate a PPI target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described above.

In yet another embodiment, the cell-free assay comprises contacting the PPI protein or biologically-active portion thereof with a known compound which binds PPI protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a PPI protein, wherein determining the ability of the test compound to interact with a PPI protein comprises determining the ability of the PPI protein to preferentially bind to or modulate the activity of a PPI target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or the membrane-bound form of PPI protein. In the case of cell-free assays comprising the membrane-bound form of PPI protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of PPI protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either PPI protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to PPI protein, or interaction of PPI protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-PPI fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or PPI protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of PPI protein binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the PPI protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated PPI protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with PPI protein or target molecules, but which do not interfere with binding of the PPI protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or PPI protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the PPI protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the PPI protein or target molecule.

In another embodiment, modulators of PPI protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of PPI mRNA or protein in the cell is determined. The level of expression of PPI mRNA or protein in the presence of the candidate compound is compared to the level of expression of PPI mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of PPI mRNA or protein expression based upon this comparison. For example, when expression of PPI mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of PPI mRNA or protein expression. Alternatively, when expression of PPI mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of PPI mRNA or protein expression. The level of PPI mRNA or protein expression in the cells can be determined by methods described herein for detecting PPI mRNA or protein.

In yet another aspect of the invention, the PPI proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos, *et al.*, 1993. *Cell* 72: 223-232; Madura, *et al.*, 1993. *J. Biol. Chem.* 268: 12046-12054; Bartel, *et al.*, 1993. *Biotechniques* 14: 920-924; Iwabuchi, *et al.*, 1993. *Oncogene* 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with PPI ("PPI-binding proteins" or "PPI-bp") and modulate PPI activity. Such PPI-binding proteins are also likely to be involved in the propagation of signals by the PPI proteins as, for example, upstream or downstream elements of the PPI pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for PPI is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a PPI-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with PPI.

In yet another aspect of the invention are methods which utilize the transgenic plants of the invention to identify PPI-interacting components via genetic screening protocols. These components can be for example, regulatory elements which modify PPI-gene expression, interacting proteins which directly modify PPI activity or interacting proteins which modify components of the same signal transduction pathway and thereby exert an effect on the expression or activity of PPI. Briefly, genetic screening protocols are applied to the transgenic plants of the invention and in so doing identify related genes which are not identified using a wild type background for the screen. For example an activation tagged library (Weigel, *et al.*, 2000. *Plant Physiol.* 122: 1003-1013), can be produced using the transgenic plants of the invention as the genetic background. Plants are then screened for altered phenotypes from that displayed by the parent plants. Alternative methods of generating libraries from the transgenic plants of the invention can be used, for example, chemical or irradiation induced mutations, insertional inactivation or insertional activation methods.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof.

### **Recombinant Expression Vectors and Host Cells**

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a PPI protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of

transporting another nucleic acid to which it has been linked. Exemplary expression vector constructs include for example the constructs of SEQ ID NO: 54-64. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication). Other vectors are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors or plant transformation vectors, binary or otherwise, which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). Examples of suitable promoters include for example constitutive promoters, ABA inducible promoters, tissue specific promoters or guard cell specific promoters. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced

into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., PPI proteins, mutant forms of PPI proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of PPI proteins in prokaryotic or eukaryotic cells. For example, PPI proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors) yeast cells, plant cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, e.g., Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual

codons for each amino acid are those preferentially utilized in *E. coli* (see, e.g., Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the PPI expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

Alternatively, PPI can be expressed in insect cells using baculovirus expression vectors.

Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In yet another embodiment, a nucleic acid of the invention is expressed in plants cells using a plant expression vector. Examples of plant expression vectors systems include tumor inducing (Ti) plasmid or portion thereof found in *Agrobacterium*, cauliflower mosaic virus (CaMV) DNA and vectors such as pBI121 .

For expression in plants, the recombinant expression cassette will contain in addition to the PPI nucleic acids, a plant promoter region, a transcription initiation site (if the coding sequence to transcribed lacks one), and a transcription termination/polyadenylation sequence. The termination/polyadenylation region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. Unique restriction enzyme sites at the 5' and 3' ends of the cassette are typically included to allow for easy insertion into a pre-existing vector. Examples of suitable promoters include promoters from plant viruses such as the 35S promoter from cauliflower mosaic virus (CaMV). Odell, *et al.*, *Nature*, 313: 810-812 (1985). and promoters from genes such as rice actin (McElroy, *et al.*, *Plant Cell*, 163-171 (1990)); ubiquitin

(Christensen, et al., Plant Mol. Biol., 12: 619-632 (1992); and Christensen, et al., Plant Mol. Biol., 18: 675-689 (1992)); pEMU (Last, et al., Theor. Appl. Genet., 81: 581-588 (1991)); MAS (Velten, et al., EMBO J., 3: 2723-2730 (1984)); maize H3 histone (Lepetit, et al., Mol. Gen. Genet., 231: 276-285 (1992); and Atanassova, et al., Plant Journal, 2(3): 291-300 (1992)), the 5'- or 3'-promoter derived from T-DNA of *Agrobacterium tumefaciens*, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Pat. No. 5,683,439), the Nos promoter, the rubisco promoter, the GRP1-8 promoter, ALS promoter, (WO 96/30530), a synthetic promoter, such as, Rsyn7, SCP and UCP promoters, ribulose-1,3-diphosphate carboxylase, fruit-specific promoters, heat shock promoters, seed-specific promoters and other transcription initiation regions from various plant genes, for example, include the various opine initiation regions, such as for example, octopine, mannopine, and nopaline.

Additional regulatory elements that may be connected to a PPI encoding nucleic acid sequence for expression in plant cells include terminators, polyadenylation sequences, and nucleic acid sequences encoding signal peptides that permit localization within a plant cell or secretion of the protein from the cell. Such regulatory elements and methods for adding or exchanging these elements with the regulatory elements PPI gene are known, and include, but are not limited to, 3' termination and/or polyadenylation regions such as those of the *Agrobacterium tumefaciens* nopaline synthase (nos) gene (Bevan, et al., Nucl. Acids Res., 12: 369-385 (1983)); the potato proteinase inhibitor II (PINII) gene (Keil, et al., Nucl. Acids Res., 14: 5641-5650 (1986) and hereby incorporated by reference); and An., et al., Plant Cell, 1: 115-122 (1989)); and the CaMV 19S gene (Mogen, et al., Plant Cell, 2: 1261-1272 (1990)).

Plant signal sequences, including, but not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell (Dratewka-Kos, et al., J. Biol. Chem., 264: 4896-4900 (1989)) and the *Nicotiana plumbaginifolia* extension gene (DeLoose, et al., Gene, 99: 95-100 (1991)), or signal peptides which target proteins to the vacuole like the sweet potato sporamin gene (Matsuka, et al., Proc. Nat'l Acad. Sci. (USA), 88: 834 (1991)) and the barley lectin gene (Wilkins, et al., Plant Cell, 2: 301-313 (1990)), or signals which cause proteins to be secreted such as that of PR1b (Lind, et al., Plant Mol. Biol., 18: 47-53 (1992)), or those which target proteins to the plastids such as that of rapeseed enoyl-ACP reductase (Verwaert, et al., Plant Mol. Biol., 26: 189-202 (1994)) are useful in the invention.

In another embodiment, the recombinant expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements



are known in the art. Especially useful in connection with the nucleic acids of the present invention are expression systems which are operable in plants. These include systems which are under control of a tissue-specific promoter, as well as those which involve promoters that are operable in all plant tissues.

Organ-specific promoters are also well known. For example, the patatin class I promoter is transcriptionally activated only in the potato tuber and can be used to target gene expression in the tuber (Bevan, M., 1986, *Nucleic Acids Research* 14:4625-4636). Another potato-specific promoter is the granule-bound starch synthase (GBSS) promoter (Visser, R.G.R., *et al.*, 1991, *Plant Molecular Biology* 17:691-699).

Other organ-specific promoters appropriate for a desired target organ can be isolated using known procedures. These control sequences are generally associated with genes uniquely expressed in the desired organ. In a typical higher plant, each organ has thousands of mRNAs that are absent from other organ systems (reviewed in Goldberg, P., 1986, *Trans. R. Soc. London* B314:343).

For in situ production of the antisense mRNA of GST, those regions of the GST gene which are transcribed into GST mRNA, including the untranslated regions thereof, are inserted into the expression vector under control of the promoter system in a reverse orientation. The resulting transcribed mRNA is then complementary to that normally produced by the plant.

The resulting expression system or cassette is ligated into or otherwise constructed to be included in a recombinant vector which is appropriate for plant transformation. The vector may also contain a selectable marker gene by which transformed plant cells can be identified in culture. Usually, the marker gene will encode antibiotic resistance. These markers include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamicin. After transforming the plant cells, those cells having the vector will be identified by their ability to grow on a medium containing the particular antibiotic. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host, preferably a broad host range prokaryotic origin of replication is included. A selectable marker for bacteria should also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers also include resistance to antibiotics such as kanamycin or tetracycline.

Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a polypeptide of the invention encoded in an open reading frame of a polynucleotide of the invention. Accordingly, the invention further provides methods for producing a polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another embodiment, the method further comprises isolating the polypeptide from the medium or the host cell.

A number of types of cells may act as suitable host cells for expression of a polypeptide encoded by an open reading frame in a polynucleotide of the invention. Plant host cells include, for example, plant cells that could function as suitable hosts for the expression of a polynucleotide of the invention include epidermal cells, mesophyll and other ground tissues, and vascular tissues in leaves, stems, floral organs, and roots from a variety of plant species, such as *Arabidopsis thaliana*, *Nicotiana tabacum*, *Brassica napus*, *Zea mays*, *Oryza sativa*, *Gossypium hirsutum* and *Glycine max*.

Alternatively, it may be possible to produce a polypeptide in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous polypeptides. If the polypeptide is made in yeast or bacteria, it may be necessary to modify the polypeptide produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain a functional

polypeptide, if the polypeptide is of sufficient length and conformation to have activity. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

A polypeptide may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed polypeptide or protein may then be purified from such culture (e.g., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the polypeptide or protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, a polypeptide or protein may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein containing a six-residue histidine tag. The histidine-tagged protein will then bind to a Ni-affinity column. After elution of all other proteins, the histidine-tagged protein can be eluted to achieve rapid and efficient purification. One or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a polypeptide. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant polypeptide. The protein or polypeptide thus purified is substantially free of other plant proteins or polypeptides and is defined in accordance with the present invention as "isolated."

### **Transformed Plants Cells and Transgenic Plants**

The invention includes protoplast, plants cells, plant tissue and plants (*e.g.*, monocots and dicots transformed with a PPI nucleic acid (*i.e.* sense or antisense), a vector containing a PPI nucleic acid (*i.e.* sense or antisense) or an expression vector containing a PPI nucleic acid (*i.e.* sense or antisense). As used herein, "plant" is meant to include not only a whole plant but also a portion thereof (*i.e.*, cells, and tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds).

The plant can be any plant type including, for example, species from the genera *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*,

*Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Zea, Avena, Hordeum, Secale, Triticum, Sorghum, Gossypium, Picea, Caco, and Populus.*

In some aspects of the invention, the transformed plant is resistant to biotic and abiotic stresses, *e.g.*, chilling stress, salt stress, water stress (*e.g.*, drought), disease, grazing pests and wound healing. Additionally, the invention also includes a transgenic plant that is resistant to pathogens such as for example fungi, bacteria, nematodes, viruses and parasitic weeds. Alternatively, the transgenic plant is resistant to herbicides or has delayed senescence. The transgenic plant has an increase in yield, productivity, biomass or ABA sensitivity. By resistant is meant the plant grows under stress conditions (*e.g.*, high salt, decreased water, low temperatures) or under conditions that normally inhibit, to some degree, the growth of an untransformed plant. Methodologies to determine plant growth or response to stress include for example, height measurements, weight measurements, leaf area, ability to flower, water use, transpiration rates and yield.

The invention also includes cells, tissues, including for example, leaves, stems, shoots, roots, flowers, fruits and seeds and the progeny derived from the transformed plant.

Numerous methods for introducing foreign genes into plants are known and can be used to insert a gene into a plant host, including biological and physical plant transformation protocols. See, for example, Miki et al., (1993) "Procedure for Introducing Foreign DNA into Plants", In: Methods in Plant Molecular Biology and Biotechnology, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 67-88 and Andrew Bent in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*.. The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, polyethylene glycol (PEG) transformation, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch, et al., Science, 227: 1229-31 (1985)), electroporation, protoplast transformation, micro-injection, flower dipping and biolistic bombardment.

### **Agrobacterium-mediated Transformation**

The most widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. *A. tumefaciens* and *A. rhizogenes*

are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectfully, carry genes responsible for genetic transformation of plants. See, for example, Kado, Crit. Rev. Plant Sci., 10: 1-32 (1991). Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber et al., supra; and Moloney, et al, Plant Cell Reports, 8: 238-242 (1989).

Transgenic *Arabidopsis* plants can be produced easily by the method of dipping flowering plants into an *Agrobacterium* culture, based on the method of Andrew Bent in, Clough SJ and Bent AF, 1998. Floral dipping: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Wild type plants are grown until the plant has both developing flowers and open flowers. The plant are inverted for 1 minute into a solution of *Agrobacterium* culture carrying the appropriate gene construct. Plants are then left horizontal in a tray and kept covered for two days to maintain humidity and then righted and bagged to continue growth and seed development. Mature seed is bulk harvested.

#### **Direct Gene Transfer**

A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4  $\mu\text{m}$ . The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes. (Sanford, et al., Part. Sci. Technol., 5: 27-37 (1987); Sanford, Trends Biotech, 6: 299-302 (1988); Sanford, Physiol. Plant, 79: 206-209 (1990); Klein, et al., Biotechnology, 10: 286-291 (1992)).

Another method for physical delivery of DNA to plants is sonication of target cells as described in Zang, et al., BioTechnology, 9: 996-996 (1991). Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, for example, Deshayes, et al., EMBO J., 4: 2731-2737 (1985); and Christou, et al., Proc. Nat'l. Acad. Sci. (USA), 84: 3962-3966 (1987). Direct uptake of DNA into protoplasts using  $\text{CaCl}_2$  precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. See, for example, Hain, et al., Mol. Gen. Genet., 199: 161 (1985); and Draper, et al., Plant Cell Physiol., 23: 451-458 (1982).

Electroporation of protoplasts and whole cells and tissues has also been described. See, for example, Donn, et al., (1990) In: Abstracts of the VIIth Intl. Congress on Plant Cell and Tissue Culture IAPTC, A2-38, page 53; D'Halluin et al., Plant Cell, 4: 1495-1505 (1992); and Spencer et al., Plant Mol. Biol., 24: 51-61 (1994).

**Particle Wounding/Agrobacterium Delivery**

Another useful basic transformation protocol involves a combination of wounding by particle bombardment, followed by use of *Agrobacterium* for DNA delivery, as described by Bidney, et al., Plant Mol. Biol., 18: 301-31 (1992). Useful plasmids for plant transformation include Bin 19. See Bevan, Nucleic Acids Research, 12: 8711-8721 (1984), and hereby incorporated by reference.

In general, the intact meristem transformation method involves imbibing seed for 24 hours in the dark, removing the cotyledons and root radical, followed by culturing of the meristem explants. Twenty-four hours later, the primary leaves are removed to expose the apical meristem. The explants are placed apical dome side up and bombarded, e.g., twice with particles, followed by co-cultivation with *Agrobacterium*. To start the co-cultivation for intact meristems, *Agrobacterium* is placed on the meristem. After about a 3-day co-cultivation period the meristems are transferred to culture medium with cefotaxime plus kanamycin for the NPTII selection.

The split meristem method involves imbibing seed, breaking of the cotyledons to produce a clean fracture at the plane of the embryonic axis, excising the root tip and then bisecting the explants longitudinally between the primordial leaves. The two halves are placed cut surface up on the medium then bombarded twice with particles, followed by co-cultivation with *Agrobacterium*. For split meristems, after bombardment, the meristems are placed in an *Agrobacterium* suspension for 30 minutes. They are then removed from the suspension onto solid culture medium for three day co-cultivation. After this period, the meristems are transferred to fresh medium with cefotaxime plus kanamycin for selection.

**Transfer by Plant Breeding**

Alternatively, once a single transformed plant has been obtained by the foregoing recombinant DNA method, conventional plant breeding methods can be used to transfer the gene and associated regulatory sequences via crossing and backcrossing. Such intermediate methods will comprise the further steps of: (1) sexually crossing the transgenic plant with a plant from a second taxon; (2) recovering reproductive material from the progeny of the cross; and (3) growing transgenic plants from the reproductive material. Where desirable or necessary, the agronomic characteristics of the second taxon can be substantially preserved by expanding this method to include the further steps of repetitively: (1) backcrossing the transgenic progeny with non-transgenic plants from the second taxon; and (2) selecting for expression of an associated

marker gene among the progeny of the backcross, until the desired percentage of the characteristics of the second taxon are present in the progeny along with the gene or genes imparting marker gene trait.

By the term "taxon" herein is meant a unit of botanical classification. It thus includes, genus, species, cultivars, varieties, variants and other minor taxonomic groups which lack a consistent nomenclature.

### **Regeneration of Transformants**

The development or regeneration of plants from either single plant protoplasts or various explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch et al., 1985). In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described (Fraley et al., 1983). In particular, U.S. Pat. No. 5,349,124 (specification incorporated herein by reference) details the creation of genetically transformed lettuce cells and plants resulting therefrom which express hybrid crystal proteins conferring insecticidal activity against Lepidopteran larvae to such plants.

This procedure typically produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants, or pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen from plants of those important lines is used to pollinate regenerated plants. A transgenic plant of the present

invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

A preferred transgenic plant is an independent segregant and can transmit the gene and its activity to its progeny. A more preferred transgenic plant is homozygous for the gene, and transmits that gene to all of its offspring on sexual mating. Seed from a transgenic plant may be grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for increased expression of the transgene.

## EMBODIMENTS

The constructs and methods of this invention have numerous applications of commercial value, especially in the prevention of desiccation of plant tissues under periods of water stress. Genetic manipulation of crop plants incorporating inhibitors of Ftase or inactivation of the gene encoding endogenous plant Ftase would allow such plants to withstand transitory environmental stress and can broaden the environments where these plants can be grown. Thus, improving tolerance of crop plants to cold, salt and drought stress, can improve the yield of the plants under such adverse conditions.

The technology described herein can also be used to alter harvesting time and harvest quality of plants. For example, overexpression of Ftase could lead to faster drying times of crops, such as corn and other grasses. Drying corn involves the use of large amounts of propane gas. Drying times of crops such as hay, which dry naturally in the fields, could be shortened, making it less likely that rain would deteriorate the crop.

In addition, inhibition of farnesylation in plants can also be used to control the senescence program of the plants so that leaves can be maintained in a green state longer and fruits can be kept immature. For example, if an antisense construct of ERA1 or CaaX box inhibitor protein construct was placed under the control of a senescence-induced promoter, the plant would induce an inhibitor of farnesylation as the senescence program was initiated, which would in turn inhibit senescence. The result would be a plant which remains green or fruits which remain immature. Thus, the plant could be kept producing a product, such as a vegetative part, flower or fruit much longer. Thus, horticulturalists could produce plants which stayed green and continued to grow even though a wild-type plant of the same variety would senesce under the same conditions. Cut flowers could be maintained longer. Or a fruit could be kept



immature, an important product for the vegetable industry where produce lifetime to market is extremely important.

Further, the inhibition of Ftase in fruits and vegetables can reduce wilting. Thus, wilting of produce during transport and shipping could be reduced. Fruits and vegetables on the grocery shelf would also require less misting to keep them fresh and flavorful, and there would be less need to wax produce such as cucumber, apples and oranges to keep them from drying out.

Less watering would also mean that fungal and bacterial attacks on the crops, or fruits and vegetables would be reduced. For example, plant diseases in the field which result from splashing of plant pathogens from the soil to the plant leaves and fruits could be inhibited.

In the field of horticulture, many drought-resistant varieties could be produced for landscaping and for use as ornamental house plants. Especially valuable would be varieties of plants which are used for potting, as ornamentals inside or outside homes and offices, and which can survive infrequent water. This would be a considerable boon for gardeners, especially during the droughty summer months where forgotten plants dry out quickly in the sun. Further, plants grown under trees and in other shady areas often experience drought conditions and limited light. The technology provided herein can provide plant varieties which can better survive under these conditions.

In a further embodiment, horticulturalists could find many uses for plants wherein lateral branching and/or flower numbers can be regulated with light/dark cycles. Examples of plants in which longer, unbranched stems would confer marketable advantage include roses, carnations, lilies, and the like. The ability to increase the number of flowers or florets on the plant is also a highly valuable asset. These traits could also be useful for many agricultural crops in that yields can be increased in a manner which also made harvesting of the crop easier.

Another benefit of the constructs and methods provided herein is that the ERA1 promoter is active in the guard cells of leaves. A portion of the ERA1 gene promoter can be fused to antisense nucleic acid to the ERA1 gene so Ftase activity is diminished only in the guard cells.

A further embodiment is the use of the drought-resistant trait as a selectable marker of transformation in plants, plant cells and plant tissues. One method of detecting transformation in plants consists of: (a) incorporating a nucleic acid construct comprising a promoter operably-linked to nucleic acid comprising antisense to SEQ ID NO:1 or nucleic acid comprising a functional equivalent or fragment thereof of the antisense; (b) inserting the nucleic acid construct into a plant, plant cell or plant tissue; (c) growing the plant, or regenerating a plant from the plant cell or plant tissue until stomates are formed; and (d) placing the plant or

regenerated plant under conditions wherein the plant is drought stressed, wherein survival of the plant under drought conditions compared to untransformed plants is indicative of transformation. Thus, this technology can be used as a selectable genetic marker, *i.e.*, a visual marker especially when combined with plant selection and transformation schemes.

In addition, without resorting to stressing a transgenic plant, the branching and/or flowering habit of plants with loss of Ftase function differs substantially from that of wild-type plants and can be used as a marker for successful transformation. This method would be especially useful where *in planta* transformation techniques have been applied. Under diurnal light conditions, shoots of transgenic plants will demonstrate less lateral branching than that of untransformed shoots, thus indicating effective loss of Ftase activity without the use of selective antibiotic markers.

## EXEMPLIFICATION

### Example 1: Mutagenesis conditions

*Arabidopsis* plants used in this study were grown under continuous light in soil- or agar-containing petri plates as described elsewhere (Haughn and Somerville 1986). Two distinct wild-types of *Arabidopsis* were used: Meyerowitz's Colombia (MCol) (Lelhe Seeds, Dripping Springs, TX) and Wassilewskija (Ws) (ABRC, Ohio State University). T-DNA mutagenized seeds were screened and mutants were isolated in the Wassilewskija background. These were obtained from the Ohio State *Arabidopsis* seed stock collection (ABRC stock numbers CS2606-2654). The T-DNA seed collection was comprised of 49 pools of 1200 fourth generation (T4) offspring derived from 100 mutagenized parents. A mutagenized parent was obtained by incubating wild-type (T1) seeds overnight in a saturating *Agrobacterium* culture containing a T-DNA plasmid carrying a gene conferring kanamycin resistance. The seeds were then washed in water and planted into pots. T2 generation seed were obtained from each plant and tested for kanamycin resistance. Kanamycin-resistant plants were advanced to the T3 generation. T4 generation plants were given to the stock center. Each pool was screened separately.

Fast neutron-irradiated seeds were screened and mutants were isolated in Meyerowitz's Columbia background. Mutagenized wild-type seeds (N1) were irradiated with 60 Gy of fast neutrons and grown to the next generation. The N2 seeds were obtained as pools of

approximately 11,000 seeds generated from 1387 N1 parents. Ten of these pools were screened separately for ABA supersensitive mutations. In the initial screen, all seeds had been stored at 4°C and were plated without imbibing. For all subsequent screens, seeds were imbibed at 4°C for one week on 0.3 µM ABA and scored for cotyledon emergence after 5-7 days at 22°C in the light.

### Example 2: Genetic Analysis

Mutant lines were backcrossed to wild type three times. T-DNA mutations were backcrossed to Ws and fast neutron mutants to MCol. Segregation of the *era* phenotype was followed by plating F2 seeds on both 0.3 µM ABA and imbibing four days at 4°C. Following imbibition, plates were transferred to room temperature in the light. Germination was measured as the presence or absence of expanded cotyledons in seedlings one week after imbibition. Double mutants were constructed by crossing lines homozygous for each mutation following segregation and identifying lines that carried one of the mutant phenotypes. The *abi3* allele used in this study is *abi3-6* (Nambara *et al.*, 1994) and the *abi1* allele is *abi1-1* (Koornneef *et al.*, 1982). The *era1-2* allele was used as the *era* parent. Segregation analysis suggested *era1* partially suppressed the insensitivity of *abi1* to ABA, so F2 plants were first screened for insensitivity to 3 mM ABA, and F3 seed from these plants were scored for sensitivity to 0.3 µM ABA. Putative *era1 abi1* double mutants were progeny-tested in the F4 generation and verified by DNA polymorphism analysis for both Era 1 and Abi1. For *era1 abi3* double mutants, F2 seeds were screened for insensitivity to 3 µM ABA, and mature plants were scored for protruding carpels and immature green seeds (Nambara *et al.*, 1994). Putative double mutant lines were also verified by DNA polymorphism analysis for both Era1 and Abi3.

### Example 3: DNA and RNA Analysis

The methods employed for DNA (Dellaporta *et al.*, 1983) and RNA (Verwoerd *et al.*, 1989) extractions were as described elsewhere. High stringency Southern blots were carried out at 65°C according to standard protocols described elsewhere (Sambrook *et al.*, 1989). All genomic and cDNA library screening was done on Gelman BioTrace NT membranes according to the manufacturer's specifications (Gelman Sciences). To clone insertion junctions between T-DNA and genomic DNA in the *era1-1* mutant (isolated from T12W DNA) a library of T12W DNA was made in  $\gamma$ -ZAPII (Stratagene). Genomic Southern blots of T12W DNA digested with restriction endonuclease *EcoR* I and probed with right border (RB) T-DNA produced three

bands (13.0 Kb, 7.0 Kb and 8.0 Kb). Subsequent analysis with additional restriction enzymes verified that the 7.0 and 8.0 Kb bands contained the insertion junctions between T-DNA and flanking plant DNA. These fragments were cloned by digesting genomic DNA with *EcoR* I, fractionating the digested DNA using a Prep Cell (Pharmacia), and identifying the fractions containing the 7.0 and 8.0 Kb by Southern blot analysis using the RB as a probe. Pooled fractions containing both the 7.0 and 8.0 Kb fragments were then ligated to the  $\gamma$ -ZAPII vector arms according to the manufacturer's instructions (Stratagene). A library containing approximately 40,000 individual recombinant bacteriophage was screened. Five positive plaques were identified and excised plasmid forms of the cloned inserts were isolated according to the manufacturer's instructions (Stratagene). Two plasmids which hybridized to the RB probe were designated pL4B and pL7 and selected for further characterization. A 2.3 kb *EcoR* I-*BamH* I restriction fragment from clone pL4B was subcloned into the plasmid pBluescript and designated pSC10. A 1.3 Kb *Hind* III- *BamH* I restriction fragment from clone pL7 was also subcloned into pBluescript and designated pSC11. Each of these plasmids contains approximately 1.2 Kb of T-DNA attached to the flanking plant genomic DNA. pSC10 was used as a probe to screen an *Arabidopsis* cDNA library called PRL2  $\lambda$ -ZipLox (ABRC, Stock CD4-7). This screen identified five positive cDNAs, and the longest cDNA insert, clone pZL23, was used to screen an additional 200,000 recombinant PRL2 phage. Subsequently a longer cDNA insert, clone pZL51, which contained an insert of 1.35 Kb, was isolated. Both cDNA clones pZL23 and pZL51 were sequenced and used to screen 30,000  $\gamma$ -ZAPII plaques made from wild-type Columbia genomic DNA partially digested with *EcoR* I. Construction of this library was as described above except the digested DNA was not size-fractionated. This screen identified four positive clones. The inserts were excised and excised plasmid forms of the cloned inserts were isolated according to the manufacturer's instructions. A 6 Kb region encompassing the entire pZL51 clone was completely sequenced. This genomic insert and a 14 Kb genomic insert isolated by screening a  $\lambda$ -FIX genomic library from *Lansberg erecta* via similar methods (ABRC Stock CD4-8) were used as probes to analyze deletion size in the fast neutron mutants *eral*-2 and *eral*-3.

#### **Example 4: Protein Farnesyl Transferase Assay**

Farnesyl transferase (Ftase) assays were performed using Ftase from cell-free extracts of wild-type and mutant plants and synthetic heptapeptides as substrate for the reaction. Peptides were purchased from Genemed Biotechnologies, Inc. The peptide sequences used were based

on the data of Randall *et al.* (1993): GGCCAIM (-CAIM) and GGCCAIL(-CAIL). Solutions of peptides were prepared in 100% dimethyl sulfoxide (DMSO) containing 10 mM dithiotreitol (DTT) and diluted in 10 mM DTT without DMSO. The cell-free extracts contained soluble protein isolated from the buds of three week old plants, either wild-type or mutant strains. First 1 g of fresh buds was collected and homogenized in a buffer containing 50 mM Hepes (pH 7.5), 1 mM MgCl<sub>2</sub>, 1 mM EGTA, 5 mM DTT, 2 µg/ml leupeptin, 2 µg/ml aprotinin, and 1 mM PMSF. Next, cellular debris and membranes were removed by centrifugation at 4°C at 10,000×g for 10 minutes and 100,000×g for 30 minutes. Following the second centrifugation, the supernatant was decanted and total soluble protein was quantified by the method of Bradford (1976). Soluble protein extracts were incubated at 30°C with a peptide substrate and radio-labeled <sup>3</sup>H-farnesyl pyrophosphate (FPP) (Amersham) for 40 minutes. Each reaction mixture contained the following components in a final volume of 25 µl: 50 mM Hepes (pH 7.5), 5 mM MgCl<sub>2</sub>, 5 mM DTT, 50 µM peptide, 0.5 µM [<sup>3</sup>H]FPP, and 100 µg of soluble protein extract. One control reaction contained soluble protein extracts that had been boiled for 5 minutes to irreversibly denature all protein. Reactions were terminated by adding EDTA to a final concentration of 50 mM and then spotted onto Silica Gel 60 thin-layer chromatography (TLC) plates (Millipore). TLC plates were developed with *n*-propanol and water (7:3 v/v) for 4-5 hours. The plates were dried, sprayed with En<sup>3</sup>Hance (New England Nuclear), and exposed to Kodak X-OMAT AR film at -70°C for 4 days.

#### **Example 5: ERA1-β-glucuronidase gene constructs and transgenic plants**

ERA1-β-glucuronidase (ERA1-GUS) fusion constructs were made by inserting a 5 Kb *EcoR* I-*Hind* III genomic fragment of the ERA1 promoter into a promoterless GUS T-DNA plasmid pBI121 containing a gene conferring resistance to the antibiotic ampicillin. This construct was then transformed into *Agrobacterium* strain LB4404. The *Agrobacterium* was grown to a density of 0.8 O.D. units (measured at 595 nm). The cells were then washed extensively in water, resuspended in sterile 10% glycerol and purified plasmid DNA encoding the ERA1-GUS fusion construct was added. Finally, the mixture of cells and DNA was pulsed in an electroporator at 200 Ohms 25 µF, 2.5 kvolts. Cells were then plated on Luria Broth agar plates containing 100 µg/ml ampicillin and grown for 2 days at 28°C. Ampicillin-resistant transformants were cultured and plasmid DNA isolated from the cultures by standard techniques was used in subsequent plant transformation experiments.

Transgenic plants were made by vacuum infiltrating plants with a saturated *Agrobacterium* culture grown to a density of 0.8 O.D. units as measured at 595 nm. Wild-type plants were grown under standard laboratory conditions (at 25°C, 150  $\mu\text{E m}^{-2} \text{ sec}^{-1}$ , humidity, constant light) until they produced their first bolts at approximately 5 weeks. Next, plant stems were removed and the plants were submerged in a solution of *Agrobacterium* and placed under a 20 mBar vacuum for 5 minutes. After the vacuum was broken, the plants were transferred to soil and allowed to recover under standard laboratory conditions as described above. After two months, the plants produced new flowers and seed which was harvested and allowed to dry for 2 weeks. Seed from individual plants were planted onto Murashige and Skoog (MS) minimal medium plates containing 50  $\mu\text{g/ml}$  kanamycin. Green kanamycin-resistant plantlets were identified and transferred to soil after 2 weeks and allowed to grow for seed. These seeds were germinated and the seedlings were tested for GUS activity using the fluorescent GUS substrate Imagen Green (Molecular Probes, Eugene, Oregon). GUS activity was assayed by suspending seedlings in GUS buffer (50 mM Sodium phosphate, pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.1% Sodium sarcosyl, 4 mM Imagen Green) for 2-4 hours in the dark at room temperature. Seedlings were viewed under a microscope at 25X magnification using blue light to generate a positive fluorescent signal. When this mixture is treated with blue light, GUS activity will produce yellow light in a background of red auto-fluorescence generated by red chlorophyll.

#### **Example 6: Drought Experiments**

Six wild-type and six *era1-2* seedlings were grown for four weeks in constant light with constant watering (25°C, 150  $\mu\text{E m}^{-2} \text{ sec}^{-1}$ , 70% humidity, constant light). The plant and pot were weighed and the pots were then covered with aluminum foil to retard soil evaporation. At this time, plants were no longer watered and each pot was weighed daily. At the end of the experiment plants were removed from the pots, which were allowed to dry for another two weeks, when they were weighed to determine the weight of the dry soil and pot. This weight was subtracted from each sample.

#### **Example 7: Age-related changes in detached leaves**

The chlorophyll content in adult rosette leaves in wild-type Columbia and *era1-2* mutants were compared after detachment from plants. The plants were grown under constant light and temperature (150  $\mu\text{E/m}^2\text{-sec}$ , 22°C) to a similar developmental age of 3 weeks after germination. At this time, the fifth leaves of several plants which had emerged after germination

were removed and placed on petri plates containing 0.8% agar with minimal salts. The plates were sealed and placed at 22°C under constant light (50  $\mu\text{E}/\text{m}^2\cdot\text{sec}$ ) for 12 days. Photographs were taken and color comparisons made at 0, 3, 6, 9, and 12 days.

**Example 8: Determination of transcript levels for selected genes in aging leaves.**

Mutant (*era1-2*) and wild-type plants were grown under constant light and temperature (150  $\mu\text{E}/\text{m}^2\cdot\text{sec}$ , 22°C) to a similar developmental age of 4 weeks after germination. At that time, the fifth rosette leaf which had emerged following germination was removed from all plants. These leaves were assayed for expression levels of three genes: *Arabidopsis* chlorophyll binding protein (CAB) and senescence-activated genes 12 and 13 (SAG12 and SAG13). mRNA transcript levels were assayed by Northern blot analysis at 0, 4, 8 days after the plants bolted. The CAB gene encodes the *Arabidopsis* chlorophyll binding protein which is involved in capturing light for photosynthesis. It is required for the green color of the leaf and is a good marker of chlorophyll turnover in the plant. CAB in wild-type plants shows transcript level reduction upon induction of senescence. No transcript level reduction was observed in aging leaves of *era1-2* mutants. SAG12 and SAG13 are *Arabidopsis* genes cloned by differential expression during senescence (SAG stands for senescence activated gene). Transcription of both genes is induced during the onset of senescence in wild-type *Arabidopsis* plants. These genes were not induced under the same developmental conditions in the *era1-2* mutants.

**Example 9: Cloning of *Arabidopsis thaliana* FTA and Construction of Transformation Vector**

The *Arabidopsis thaliana* FTA sequence was obtained by RT-PCR from total RNA isolated from leaf tissue using primers corresponding to SEQ ID NO:17 and SEQ ID NO:18. The resulting fragment was digested with *Bam*HI and *Sma*I and cloned into the plasmid pCR2.1. The Clontech vector pBI121 was used as the backbone for the antisense construct. The GUS gene was removed by *Bam*HI and *Eco*1CRI digestion and replaced with the FTA insert that was cut from pCR2.1-FTA using *Sma*I and *Bam*HI and ligated into the vector SEQ ID NO: 10.

**Table 1.**

SEQ ID NO:17: 5' - AAAGGATCCTCAAATTGCTGCCACTGTAAT -3'  
 SEQ ID NO:18: 5' - AAACCCGGGATGAATTTTCGACGAGAACGTG -3'

**Example 10: Cloning of non-full length *Brassica napus* FTA and FTB nucleic acid sequences**

RNA was isolated from leaf and root tissue using the Qiagen RNeasy kit. RT-PCR was performed by known techniques using the primers shown in Table 2. The FTA sequence was obtained using the primer pair SEQ ID NO:25 and SEQ ID NO:26. The FTB sequence was obtained using the primer pair SEQ ID NO:27 and SEQ ID NO:28.

**Table 2.**

SEQ ID NO:25:	5' -GGATCCATGGATTACTTCCGTGCGATTTACTTCTCC-3'
SEQ ID NO:26:	5' -AAAAAGCTTCCATGCCCAATAGTTAGCTCTTATTGGATC-3'
SEQ ID NO:27:	5' -AAAAAGCTTTGGCTTTGTTACTGGATTCTTCATTCAAT-3'
SEQ ID NO:28:	5' -AAATCTAGAAGCTTCATAATACCGATCCAAGACAATGTT-3'

PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the cloning vector pBluescript KS+. The vector was digested with *EcoRV* and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5 $\alpha$  cells, positive colonies were selected and the resulting inserts sequenced.

**Example 11: Cloning of non-full length FTA and FTB nucleic acid sequences from *Glycine max* and *Zea maize***

RNA was isolated from leaf and root tissue using the Qiagen RNeasy kit. RT-PCR was performed by known techniques using the primers shown in Table 3. The *Glycine max* FTA sequence was obtained using the primer pair SEQ ID NO:29 and SEQ ID NO:30. The *Glycine max* FTB sequence was obtained using the primer pair SEQ ID NO:31 and SEQ ID NO:32. The *Zea maize* FTB sequence was obtained using the primer pair SEQ ID NO:33 and SEQ ID NO:34.

**Table 3.**

SEQ ID NO:29:	5' -AAAGGATCCATGGAATCTGGGTCTAGCGA-3'
SEQ ID NO:30:	5' -AAATCTAGAAGGAAGTCTGCTCTTGCGC-3'
SEQ ID NO:31:	5' -AAATCTAGAGCCACCATTCTCGCAACG-3'
SEQ ID NO:32:	5' -AAAGAGCTCGTGGTGGAGAATCTGGGTGC-3'
SEQ ID NO:33:	5' -GGCGGATCCCGACCTACCGAGG-3'



SEQ ID NO:34: 5' -AAAGAGCTCGTGGATGGATTGGCTCCAGC- 3'

PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the cloning vector pBluescript KS+. The vector was digested with *EcoRV* and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5 $\alpha$  cells, positive colonies were selected and the resulting inserts sequenced.

### Example 12: Sequence Analysis

#### *Arabidopsis thaliana* FTA

A disclosed nucleic acid of 999 nucleotides (also referred to as FT1) is shown in Table 4A. The primers used in the PCR are depicted in bold.

<b>Table 4A. FT1 Nucleotide Sequence (SEQ ID NO:7).</b>
<b>Aa</b> acccgggatgaatttc <b>gacgag</b> accgtgccactgagccaacgattggagtggtcagacgtggt cccattgactcaggacgatgggtccgaatccagtggtgccaattgcctacaaggaagagttccgcg agactatggattacttccgtgcgatttacttttccgacgagcgatctcctcgcgactacgactc acggaagaaacccctcctcttaactccggcaactacacagtggtggtcatttcaggcgcttagtact cgaggcccttaatcacgacttggttgaagaactcgagttcatcgaaacgatttgctgaggataact ctaagaactaccaactgtggcatcatcgcgagtggttgcagagaaaactgggtcctgatgttgca gggagagaacttgaatttaccgtagagtactttcacttgatgccaacattatcatgcttggtc acataggcagtggtgacactacgggcattaggaggatgggaagatgagctcgattactgtcacgagc tccttgaagctgacgtctttaacaattccgcctggaatcagaggtattatgtcatcacccaatct cctttgttgggaggcctagaagccatgagagaatctgaagtaagctacacaatcaaagccatttt aaccaatcctgcaaacgagagctcatggcgatacctaaaagcgctttacaaagacgacaaagaat cctggattagtgatccaagtgtttcctcagtcgttgaatgttctatcccgcacagattgcttc catggattcgctctgagcacccttttgatcttctatgtgatggactgagaccaaccaacgagca taaagactcagtgagagctctagctaataagaaccagagactaacttggccaatttggtgtgta ctattcttgggtcggtgtagatcctataagagctaactattgggcatggaggaagagcaagatt <b>aca</b> <b>gtggcagcaatttgaggatccttt</b>

A disclosed FT1 polypeptide (SEQ ID NO:11) encoded by SEQ ID NO:7 has 326 amino acid residues and is presented in Table 4B using the one-letter amino acid code.

<b>Table 4B. Encoded FT1 protein sequence (SEQ ID NO:11).</b>
MNFDETVPLSQRLWSDVVPLTQDDGPNPVVPIAYKEEFRETMDYFRAIYFSDERSPRALRLTE ETLLLNSGNYTVWHFRRLVLEALNHDLFEELEFIERIAEDNSKNYQLWHHRRWVAEKLGPDVAG RELEFTRRVLSLDAKHYHAWSHRQWTLRALGGWEDEL DYCHELLEADVFNNSAWNQRYYVITQS PLGGGLEAMRESEVSYTIKAILTNPANESSWRYLKALYKDDKESWISDPSVSSVCLNVLSRTDC FHGFALSTLLDLLCDGLRPTNEHKDSVRALANEPEPTNLANLVCTILGRVDPIRANYWAWRKS ITVAAI

Due to the nature of the cloning strategy the sequence presented does not contain any 5' or 3' non-translated sequence. Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the

rapid amplification of cDNA ends (RACE) technology or other such PCR techniques. The percent identity of the *Arabidopsis thaliana* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 17.

The present invention also includes a nucleic acid sequence complimentary to the *Arabidopsis thaliana* farnesyl transferase alpha subunit of SEQ ID NO:7. The disclosed complementary sequence is shown as SEQ ID NO:8. The nucleic acid sequence of SEQ ID NO:9 shows the nucleic acid sequence of SEQ ID NO:8 that has been prepared for ligation into an expression vector.

#### SEQ ID NO:8

**aaaggatcctcaaattgctgccactgtaatcttgctcttcctccatgccaatagttagctctt  
ataggatctacacgaccaagaatagtacacaccaaattggccaagttagttctctggttcttcat  
tagctagagctctcactgagtcctttatgctcgttgggttggtctcagtcctcatcacatagaagatc  
caaaaggggtgctcagagcgaatccatggaagcaatctgtgcgggatagaacattcaaacagact  
gaggaaacacttggtacctaataccaggattctttgtcgtctttgtaaagcgcttttaggtatc  
gcatgagctctcgtttgcaggattggttaaaatggctttgattgtgtagcttacttcagattc  
tctcatggcttctaggcctcccaacaaaggagattgggtgatgacataatacctctgattccag  
gcggaattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatcttcccatc  
ctcctaatagcccgtagtggtccactgcctatgtgaccaagcatgataatgtttggcatcaagtga  
aagtactctacgggtaaattcaagttctctccctgcaacatcaggacccagtttctctgcaacc  
catcgccgatgatgccacagttggttagttcttagagttatcctcagcaatgcgttcgatgaact  
cgagttcttcaacaagtcgtgattaagggcctcgagtactaggcgctgaaatgccacactgt  
gtagttgccggagtttaagaggagggtttcttccgtgagtcgtagtgccgaggagatcgctcg  
tcggaaaagtaaatcgacggaagtaatccatagtcctcgcggaactcttcttgttaggcaattg  
gcaccactggattcggaccatcgtcctgagtcgaatgggaccacgtctgaccactccaatcgttg  
gctcagtgggcacgggtctcgtcgaaattcatccggggttt**

#### SEQ ID NO:9

**gatcctcaaattgctgccactgtaatcttgctcttcctccatgccaatagttagctcttatag  
gatctacacgaccaagaatagtacacaccaaattggccaagttagttctctggttcttcattagc  
tagagctctcactgagtcctttatgctcgttgggttggtctcagtcctcatcacatagaagatccaaa  
aggggtgctcagagcgaatccatggaagcaatctgtgcgggatagaacattcaaacagactgagg  
aaacacttggtacctaataccaggattctttgtcgtctttgtaaagcgcttttaggtatcgcca  
tgagctctcgtttgcaggattggttaaaatggctttgattgtgtagcttacttcagattctctc  
atggcttctaggcctcccaacaaaggagattgggtgatgacataatacctctgattccaggcgg  
aattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatcttcccatcctcc  
taatgcccgtagtggtccactgcctatgtgaccaagcatgataatgtttggcatcaagtgaaggt  
actctacgggtaaattcaagttctctccctgcaacatcaggacccagtttctctgcaaccatc  
gccgatgatgccacagttggttagttcttagagttatcctcagcaatgcgttcgatgaactcgag  
ttcttcaacaagtcgtgattaagggcctcgagtactaggcgctgaaatgccacactgtgtag  
ttgccggagtttaagaggagggtttcttccgtgagtcgtagtgccgaggagatcgctcgtcgg  
aaaagtaaatcgacggaagtaatccatagtcctcgcggaactcttcttgttaggcaattggcac  
cactggattcggaccatcgtcctgagtcgaatgggaccacgtctgaccactccaatcgttggtc  
agtgggcacgggtctcgtcgaaattcatccc**

**Brassica napus FTA**

A disclosed nucleic acid of 822 nucleotides (also referred to as FT2) is shown in Table 5A.

<b>Table 5A. FT2 Nucleotide Sequence (SEQ ID NO:12).</b>
ATGGATTACTTCCGTGCGATTACTTCTCCGACGAGCGTTCTGCTCGCGCGCTGCGACTCACGGA AGAAGCTCTCCGCTTAACTCGGGCAACTACACCGTGTGGCACTTCGGGCGCTTAGTACTCGAGG AGCTTAATAACGACTTGTATGAAGAGCTCAAGTTCATCGAAAGCATTGCTGAGGATAACTCTAAG AACTACCAGTTGTGGCATCATCGACGATGGGTGCGAGAGAACTGGGTCCTGATGTTGCAGGAAA GGAAGTTGAGTTTACTCGGAGGGTACTATCACTTGATGCCAAGCATTATCATGCTTGGTCACATA GGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAAATGAGCTTAACTACTGCCACGAGCTCCTT GAAGCTGACGCTCTTAACAACCTCTGCATGGAATCAGAGGTATTACGTTATAACTAGATCACCTTC GTTGGGAGGCCTAGAAGCCATGAGAGAATCTGAAGTAAGCTACACAGTCAAAGCCATTTTAGCAA ATCCCGGGAACGAGAGCTCTTGGAGGTACCTGAAAGCCCTTTACAAAGACGACACAGAGTCTTGG ATTAGTGATCCAAGTGTTTCTCAGTCTGTTTGAAGTTCTCTCACGCGCGGACTGCTTCCATGG ATTCGCTCTGAGCACCCCTTTTGGATCTTCTGTGCGATGGGTTGAGACCAACCAACGAGCATAGAG ACTCGGTGAAAGCTCTAGCTAATGAAGAACCAGAGACTAACTTGGCCAATTTGGTGTGTACCATT CTGTGTCGTGTTGATCCAATAAGAGCTAACTATTGGGCATGG

A disclosed FT2 polypeptide (SEQ ID NO:13) encoded by SEQ ID NO:12 has 274 amino acid residues and is presented in Table 5B using the one-letter amino acid code.

<b>Table 5B. Encoded FT2 protein sequence (SEQ ID NO:13).</b>
MDYFRAIYFSDERSARALRLTEELRLNSGNYTVWHFGRVLVLEELNNDLYEELK FIESIAEDNSKNYQLWHHRRWVAEKLGPDVAGLEKEFTRRVLSLDAKHYHAWSH RQWALQALGGWENELNYCHELLEADVFNNSAWNQRYVITRSPSLGGLEAMRES EVSYTVKAILANPGNESSWRYLKALYKDDTESWISDPSVSSVCLKVLSRADCFH GFALSTLLDLLCDGLRPTNEHRDSVKALANEEPETNLANLVCTILCRVDPIRAN YWAWKL

Due to the nature of the cloning strategy the sequence presented is not full length. Compared to the *Arabidopsis thaliana* sequence there are 42 amino acids missing from the amino terminus and 10 amino acids from the carboxy terminus. The percent identity of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napsus* farnesyl transferase alpha subunit of SEQ ID NO:12. The disclosed complimentary sequence is shown as SEQ ID NO:35.

SEQ ID NO:35

CCATGCCCAATAGTTAGCTCTTATTGGATCAACACGACACAGAATGGTACACACCAAATTGGCC  
 AAGTTAGTCTCTGGTTCTTCATTAGCTAGAGCTTTACCGAGTCTCTATGCTCGTTGGTTGGTC  
 TCAACCCATCGCACAGAAGATCCAAAAGGGTGCTCAGAGCGAATCCATGGAAGCAGTCCGCGCG  
 TGAGAGAACTTTCAAACAGACTGAGGAAACACTTGGATCACTAATCCAAGACTCTGTGTCTGTCT  
 TTGTAAAGGGCTTTAGGTACCTCCAAGAGCTCTCGTTCCCGGGATTTGCTAAAATGGCTTTGA  
 CTGTGTAGCTTACTTCAGATTCTCTCATGGCTTCTAGGCCTCCCAACGAAGGTGATCTAGTTAT  
 AACGTAATACCTCTGATTCCATGCAGAGTTGTTAAAGACGTCAGCTTCAAGGAGCTCGTGGCAG  
 TAGTTAAGCTCATTTTTCCCATCCTCCTAATGCTTGTAGCGCCCACTGCCTATGTGACCAAGCAT  
 GATAATGCTTGGCATCAAGTGATAGTACCCTCCGAGTAAACTCAAGTTCCTTTCTGCAACATC  
 AGGACCCAGTTTCTCTGCGACCCATCGTCGATGATGCCACAACCTGGTAGTTCTTAGAGTTATCC  
 TCAGCAATGCTTTCGATGAACCTTGAGCTCTTCATACAAGTCGTTATTAAGCTCCTCGAGTACTA  
 AGCGCCCGAAGTGCCACACGGTGTAGTTGCCCGAGTTTAAGCGGAGAGCTTCTTCCGTGAGTCG  
 CAGCGCGCGAGCAGAACGCTCGTCGGAGAAGTAAATCGCACGGAAGTAATCCAT

### Brassica napus FTB

A disclosed nucleic acid of 1110 nucleotides (also referred to as FT3) is shown in Table 6A.

Table 6A. FT3 Nucleotide Sequence (SEQ ID NO:14).
TGGCTTTGTACTGGATTCTTCATTCAATTGCTTTGCTTGGGGAGTCTGTGGATGATGACTTAGA AAACAATGCAATCGATTTTCTTGGACGTTGCCAGGGTTCTGATGGTGGATATGGTGGTGGTCCTG GCCAACTTCCACATCTTGCAACAAGTTATGCTGCAGTGAATACACTTGTTACTTTAGGAGGTGAG AAAGCCTTCTCTTCAATTAACAGAGAACAATGGCTTGTTTCTTAAGACGAATGAAGGATACAAA TGGAGGTTTCAGGATGCATAATATGGGAGAAATAGATGTGCGAGCGTGCTACACTGCGATTTTGA TTGCAAGCATCCTGAACATTGTGGATGATGAACTCACCCGCGGCTTAGGAGATTACATTTTGAGT TGCCAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGAAGCTCATGGTGGGTACACGTA CTGTGGGTTGGCTACTATGATTTTAATCAATGAAGTCGACCGCTTGAATTTGGATTCTGTTAATGA ATTGGGTTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAGGACGAACAAATTGGTCGAC GGTGCTACACGTTTTTGGCAGGCAGCCCCCTGTGTTCTACTACAGCGATTTTTTTCATCCCAGGA TATGGCACCTCATGGATCATCATCACATATGTCACAAGGGACAGATGAAGATCACGAGGAACATG GTCATGATGAAGATGATCCTGAAGACAGTGATGAAGATGATTCTGATGAGGATAGCGATGAAGAT TCAGGGAATGGTCACCAAGTTCATCATACGCTCTACCTACATTGACAGGAGAATTCAACCTGTTTT TGATAGCCTCGGCTTGCAAAGATATGTGCTCTTGCTCTCAGGTTGCTGATGGTGGATTACAGAG ACAAGCTGAGGAAACCCCGTGACTTCTACCACATGTTACTGCCTAAGCGGTCTTTCCGTGGCT CAACACGCTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGTGACATTTTGGGTGGCTACGC AAACCACCTGAACCTGTTACCTCCTCCACAACATTGTCTTGGATCGGTATTATGAAGCTTCTA GATT

A disclosed FT3 polypeptide (SEQ ID NO:15) encoded by SEQ ID NO:13 has 370 amino acid residues and is presented in Table 6B using the one-letter amino acid code.

Table 6B. Encoded FT3 protein sequence (SEQ ID NO:15).
WLCYWILHSIALLGESVDDDLNNAIDFLGRCQGSDDGGYGGGPGQLPHLATSYA AVNTLVTLGGEKAFSSINREQMACFLRRMKDTNNGFRMHNMGEDVDRACYTAIL IASILNIVDDELTRGLGDYILSCQTYEGGIGGEPGSEAHGGYTYCGLATMILIN EVDRNLNDSL MNWVVRQGVEMGFQGR TNKLV DGCYTFWQAAPCVLLQRFSSQ DMAPHGSSSHMSQGTDEDHEEHGHDEDDPEDSDEDDSDSDSGNGHQVHHT STYIDRRIQPVFDSLGLQRYVLLCSQVADGGFRDKLRKPRDFYHTCYCLSGLSV AQHAWSKDEDTPLTRDILGGYANHLEPVHLLHNILVDRYYEASRF

Due to the nature of the cloning strategy the sequence presented is not full length. Compared to the *Arabidopsis thaliana* sequence there are 31 amino acids missing from the amino terminus and 5 amino acids from the carboxy terminus. The percent identity of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of published sequences is shown in Figure 18.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques. Sequence comparisons have been performed and percent identities are shown in Figure 17 and Figure 18.

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napsus* farnesyl transferase beta subunit of SEQ ID NO:14. The disclosed complimenary sequence is shown as SEQ ID NO:36.

#### SEQ ID NO:36

```
AAATCTAGAAGCTTCATAATACCGATCCAAGACAATGTTGTGGAGGAGGTGAACAGGTTCAAGG
TGGTTTGC GTAGCCACCCAAAATGTCACGAGTCAAAGGAGGAGTGTCTCGTCTTTTGACCAAG
CGTGTTGAGCCACGGAAGACCGCTTAGGCAGTAACATGTGTGGTAGAAGTACGCGGGTTTCCT
CAGCTTGTCTCTGAATCCACCATCAGCAACCTGAGAGCACAAGAGCACATATCTTTGCAAGCCG
AGGCTATCAAAAACAGGTTGAATTCTCCTGTCAATGTAGGTAGACGTATGATGAACTTGGTGAC
CATTCCCTGAATCTTCATCGCTATCCTCATCAGAATCATCTTCATCACTGTCTTCAGGATCATC
TTCATCATGACCATGTTCTCTCGTGATCTTCATCTGTCCCTTGTGACATATGTGATGATGATCCA
TGAGGTGCCATATCCTGGGATGAAAAAATCGCTGTAGTAGAACACAGGGGGCTGCCTGCCAAA
ACGTGTAGCAACCGTCGACCAATTTGTTCTGTCCTACCTTGGAATCCCATTTCTACTCCTTGTCTG
ATGTACAACCCAATTCAATTAACGAATCCAAATTCAAGCGGTCGACTTCATTGATTAAATCATA
GTAGCCAACCCACAGTACGTGTACCCACCATGAGCTTCGGAGCCAGGTTCCCCTCCAATGCCAC
CTTCATAAGTTTGGCAACTCAAAATGTAATCTCCTAAGCCGCGGTGAGTTCATCATCCACAAT
GTTTCAAGATGCTTGCAATCAAAATCGCAGTGTAGCACGCTCGCACATCTATTTCTCCCATATTA
TGCATCCTGAAACCTCCATTTGTATCCTTCATTCTTAAAGAAACAAGCCATTTGTTCTCTGT
TAATTGAAGAGAAGGCTTTCTCACCTCCTAAAGTAACAAGTGATTCACTGCAGCATAACTTGT
TGCAAGATGTGGAAGTTGGCCAGGACCACCACCATATCCACCATCAGAACCCTGGCAACGTCCA
AGAAAATCGATTGCATTGTTTTCTAAGTCATCATCCACAGACTCCCCAAGCAAAGCAATTGAAT
GAAGAATCCAGTAACAAAGCCA
```

#### Glycine max FTA

A disclosed nucleic acid of 1041 nucleotides (also referred to as FT4) is shown in Table 7A.

**Table 7A. FT4 Nucleotide Sequence (SEQ ID NO:37).**

```

ATGGAATCTGGGTCTAGCGAAGGAGAAGAGGTGCAGCAACGCGTGCCGTTGAGGGAGAGAGTGGA
GTGGTCAGATGTTACTCCGGTTCCTCAAAACGACGGCCCTAACCTGTCGTTCCGATCCAGTACA
CTGAAGAGTTTTCCGAAGTTATGGATTACTTTCGCGCCGTTTACCTCACCAGATGAACGCTCCCT
CGCGCCCTCGCTCTCACAGCCGAAGCCGTTCAATTCAACTCCGGCAACTACACTGTGTGGCATTT
CCGACGGTTGTTACTTGAGTCGCTAAAAGTCGACTTGAACGATGAACTGGAGTTTGTGGAGCGTA
TGGCCGCTGGAAATTCTAAAAATTATCAGATGTGnATGTTCTGTAGGCATCCTAGACGATGGGT
GCCGAGAAGTTAGGTCCTGAAGCTAGAAACAATGAGCTCGAGTTCACCAAAAAGATACTGTCCGT
TGATGCCAAACATTATCATGCATGGTCTCATAGACAGTGGGCTCTTCAAACACTAGGAGGATGGG
AAGATGAACTTAATTATTGCACAGAACTACTTAAAGAAGACATTTTTTAACAATTCTGCTTGGAAT
CAGAGATATTTTGTGATAACAAGGTCTCCTTTCTTGGGGGGCCTAAAAGCTATGAGAGAGTCTGA
AGTGCTTTACACCATCGAAGCCATTATAGCCTACCCTGAAAATGAAAGCTCGTGGAGATATCTAC
GAGGACTTTATAAAGGTGAACTACTTCATGGGTAAATGATCCTCAAGTTTCTTCAGTATGCTTA
AAGATTTTGAGAACTAAGAGCAACTACGTGTTTGCTCTTAGCACTATTTTAGATCTTATATGCTT
TGGTTATCAACCAAATGAAGACATTAGAGATGCCATTGACGCCTTAAAGACCGCAGATATGGATA
AACAAAGATTTAGATGATGATGAGAAAGGGGAACAACAAAATTTAAATATAGCACGAAATATTTGT
TCTATCCTAAACAAGTTGATCCAATTAGAACCAACTATTGGATTGGCGCAAGAGCAGACTTCC
T

```

A disclosed FT4 polypeptide (SEQ ID NO:39) encoded by SEQ ID NO:37 has 347 amino acid residues and is presented in Table 7B using the one-letter amino acid code.

**Table 7B. Encoded FT4 protein sequence (SEQ ID NO:39).**

```

MESGSSEGEVQQRVPLRERVEVSDVTPVPQNDGPNPVPVPIQYTEEFSEVMDYF
RAVYLTDERSPRALALTAEAVQFNSGNYTVWHFRRLLESCLKVDLNDLEFVER
MAAGNSKNYQMXMFCRHRPRRWVAEKLGPARNNELEFTKKILSVDKHYHAWSH
RQWALQTLGGWEDELNYCTELLKEDI FNNSAWNQRYFVITRSPFLGGLKAMRES
EVLYTIEAIIAYPENESSWRYLRGLYKGETTSWVNDPQVSSVCLKILRTKSNIYV
FALSTILDLCFGYQPNEDIRDAIDALKTADMDKQDLDDDEKGEQQNLNIARNI
CSILKQVDPIRTNYWIWRKSRLP

```

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* alpha subunit of SEQ ID NO:37. The disclosed complimentary sequence is shown as SEQ ID NO:38.

SEQ ID NO:38

```

AGGAAGTCTGCTCTTGCGCCAAATCCAATAGTTGGTTCTAATTGGATCAACTTGTTTTAGGATA
GAACAAATATTTTCGTGCTATATTTAAATTTTGTGTTCCCCTTTCTCATCATCATCTAAATCTT
GTTTATCCATATCTGCGGTCTTTAAGGCGTCAATGGCATCTCTAATGTCTTCATTTGGTTGATA

```

ACCAAAGCATATAAGATCTAAAATAGTGCTAAGAGCAAACACGTAGTTGCTCTTAGTTCTCAAA  
 ATCTTTAAGCATACTGAAGAACTTGAGGATCATTTACCCATGAAGTAGTTTCACCTTTATAAA  
 GTCCTCGTAGATATCTCCACGAGCTTTCATTTTCAGGGTAGGCTATAATGGCTTCGATGGTGTA  
 AAGCACTTCAGACTCTCTCATAGCTTTTAGGCCCCCAAGAAAGGAGACCTTGTTATGACAAAA  
 TATCTCTGATTCCAAGCAGAATTGTTAAAAATGTCTTCTTTAAGTAGTTCTGTGCAATAATTAA  
 GTTCATCTTCCCATCCTCCTAGTGTTTGAAGAGCCCACTGTCTATGAGACCATGCATGATAATG  
 TTTGGCATCAACGGACAGTATCTTTTGGTGAACCTCGAGCTCATTTGTTTCTAGCTTCAGGACCT  
 AACTTCTCGGCAACCCATCGTCTAGGATGCCTACAGAACATNCACATCTGATAATTTTTAGAAT  
 TTCCAGCGGCCATACGCTCCACAACTCCAGTTCATCGTTCAAGTCGACTTTTAGCGACTCAAG  
 TAACAACCGTCGGAATGCCACACAGTGTAGTTGCCGGAGTTGAATTGAACGGCTTCGGCTGTG  
 AGAGCGAGGGCGCGAGGGGAGCGTTCATCGGTGAGGTAAACGGCGCGAAAGTAATCCATAACTT  
 CGGAAAACCTCTTCAGTGTACTGGATCGGAACGACAGGGTTAGGGCCGTCGTTTTGAGGAACCGG  
 AGTAACATCTGACCACTCCACTCTCTCCCTCAACGGCACGCGTTGCTGCACCTCTTCTCCTTCG  
 CTAGACCCAGATTCCAT

### Glycine max FTB

A disclosed nucleic acid of 1035 nucleotides (also referred to as FT5) is shown in Table 8A.

Table 8A. FT5 Nucleotide Sequence (SEQ ID NO:40).
GCCACCATTCTCGCAACGCCCAAACCCTCATGTTGGAGCTTCAACGCGATAATCACATGCAGTA TGTCTCCAAAGGCCTTCGCCATCTCAGTTCGCGATTTTCCGTTTGGACGCTAATCGACCCTGGC TCTGCTACTGGATCTTCCACTCCATTGCTTTGTTGGGAGAATCCGTCGATGATGAACTCGAAGAT AACGCTATCGATTTTCTTAACCGTTGCCAGGATCCGAATGGTGGATATGCCGGGGGACCAGGCCA GATGCCTCATATTGCCACAACCTTATGCTGCTGTTAATTCACTTATTACTTTGGGTGGTGAGAAAT CCCTGGCATCAATTAATAGAGATAAACTGTATGGGTTTCTGCGGCGGATGAAGCAACCAATGGT GGATTACAGGATGCATGATGAAGGTGAAATTGATGTTGAGCTTGCTACACTGCCATTTCTGTTGC AAGTGTTTTGAACATTTTGGATGATGAGCTGATCCAGAATGTTGGAGACTACATTATAAGCTGTC AAACATATGAGGGTGGCATTGCTGGTGAGCCTGGTTCTGAGGCTCATGGTGGGTACACCTTTTGT GGATTAGCTACAATGATTCTGATTGGTGAGGTAACTCACTGGATCTGCCTCGATTAGTTGACTG GGTGGTATTCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAAATAAACTGGTGGATGGAT GCTATTCCTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAAGATTATCTTCTATTATCAACAAA CAGATGGAAGAGACATCACAGATTTTTCGGGTATCTTATGTATCTGAAGCAAAAGAAAGTTTGA TGAACCTCTAGTCATGCAACATGCCGTGGTGAGCATGAAGGCACCAAGTGAATCCAGTTCATCTG ATTTTAAAAATATTGCCTATAAATTTATTAATGAGTGGAGAGCACAAAGAACCACTTTTTACAGT ATTGCTTTACAGCAATATATTCTCTTATGTGCACAGGAGCAAGAGGGTGGACTGAGAGACAAACC GGGTAAACGTAGAGATCATTATCACACATGTTACTGTTTAAAGTGGACTCTCATTGTGCCAGTATA GTTGGTCAAAGCACCCAGATTCTCCACCAC

A disclosed FT5 polypeptide (SEQ ID NO:42) encoded by SEQ ID NO:40 has 378 amino acid residues and is presented in Table 8B using the one-letter amino acid code.

Table 8B. Encoded FT5 protein sequence (SEQ ID NO:42).
ATIPRNAQTLMLELQRDNMQYVSKGLRHLSSAFSVLDANRPWLCYWIFHSIAL LGESVDELEDNAIDFLNRCQDPNGGYAGGPGQMPHIATTYA AVNSLITLGGEK SLASINRDKLYGFLRRMKQPNGGFRMHDEGEIDVRACYTAISVASVLNILDDEL IQNVGDYIISCQTYEGGIAGEPGSEAHGGYTFCGLATMILIGEVNHLDLPLRLVD WVVFQKQKECGFQGRNKLVDGCYSFWQGGAVALLQRLSSIINKQMEETSQIFA VSYVSEAKESLDGTSSHATCRGEHEGTSESSSSDFKNIA YKFINEWRAQEPLFH SIALQQYIILLCAQEQEGGLRDKPGKRRDHYHTCYCLSGLSLCQYSWSKHPDSP

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* beta subunit of SEQ ID NO:40. The disclosed complementary sequence is shown as SEQ ID NO:41.

#### SEQ ID NO:41

```
GTGGTGAGAAATCTGGGTGCTTTGACCAACTATACTGGCACAATGAGAGTCCACTTAAACAGTA
ACATGTGTGATAATGATCTCTACGTTTACCCGGTTTGTCTCTCAGTCCACCCTCTTGCTCCTGT
GCACATAAGAGAATATATTGCTGTAAAGCAATACTGTGAAAAAGTGGTTCTTGCTCTCCACT
CATTAATAAATTTATAGGCAATATTTTAAATCAGATGAACTGGATTCACTGGTGCCTTCATG
CTCACCACGGCATGTTGCATGACTAGAGGTTCCATCCAACTTTCTTTTGCTTCAGATACATAA
GATACCGCAAAATCTGTGATGTCTCTTCCATCTGTTTGTTGATAATAGAAGATAATCTTTGCA
ATAGAGCAACAGCACCTCCCTGCCAAAAGGAATAGCATCCATCCACCAGTTTATTTGTTCTCCC
CTGGAATCCACATTCCTTACCTTGTCGGAATACCACCCAGTCAACTAATCGAGGCAGATCCAAG
TGATTAACCTCACCAATCAGAATCATTGTAGCTAATCCACAAAAGGTGTACCCACCATGAGCCT
CAGAACCAGGCTCACCAGCAATGCCACCCTCATATGTTTGACAGCTTATAATGTAGTCTCCAAC
ATTCTGGATCAGCTCATCATCCAAAATGTTCAAAACACTTGCAACAGAAATGGCAGTGTAGCAA
GCTCGAACATCAATTTACCTTCATCATGCATCCTGAATCCACCATTTGGTTGCTTCATCCGCC
GCAGAAACCCATACAGTTTATCTCTATTAATTGATGCCAGGGATTTCTCACCACCCAAAGTAAT
AAGTGAATTAACAGCAGCATAAGTTGTGGCAATATGAGGCATCTGGCCTGGTCCCCCGGCATAT
CCACCATTGGATCCTGGCAACGGTTAAGAAAATCGATAGCGTTATCTTCGAGTTCATCATCGA
CGGATTCTCCCAACAAAGCAATGGAGTGGAAGATCCAGTAGCAGAGCCAGGGTTCGATTAGCGTC
CAAAACGGAAAATGCGGAACTGAGATGGCGAAGGCCTTTGGAGACATACTGCATGTGATTATCG
CGTTGAAGCTCCAACATGAGGGTTTGGGCGTTGCGAGGAATGGTGGC
```

#### **Zea maize FTB**

A disclosed nucleic acid of 1235 nucleotides (also referred to as FT6) is shown in Table 9A.



**Table 9A. FT6 Nucleotide Sequence (SEQ ID NO:43).**

```

GGCGGATCCCGACCTACCGAGGCTCACGGTGACGCAGGTGGAGCAGATGAAGGTGGAGGCCAGGG
TTGGCGACATCTACCGCTCCCTCTTCGGGGCCGCGCCCAACACGAAATCCATCATGCTAGAGCTG
TGGCGTGATCAGCATATCGAGTATCTGACGCCTGGGCTGAGGCATATGGGACCAGCCTTTCATGT
TCTAGATGCCAATCGCCCTTGGCTATGCTACTGGATGGTTCATCCACTTGCTTTGCTGGATGAAG
CACTTGATGATGATCTTGAGAATGATATCATAGACTTCTTAGCTCGATGTCAGGATAAAGATGGT
GGATATAGTGGTGGACCTGGACAGTTGCCTCACCTAGCTACGACTTATGCTGCTGTAAATACACT
TGTGACAATAGGGAGCGAAAGAGCATTGTCAATCAATAGGGGCAACCTGTACAATTTTATGC
TGCAGATGAAAGATGTATCAGGTGCTTTCAGAATGCATGATGGTGGCGAAATTGATGTCCTGTCT
TCCTACACCGCTATATCGGTTGCCAGCCTTGTGAATATTCTTGATTTTAACTGGCAAAAGGTGT
AGGCGACTACATAGCAAGATGTCAAACCTATGAAGGTGGTATTGCTGGGGAGCCTTATGCTGAAG
CACATGGTGGGTATACATTCTGTGGATTGGCTGCTTTGATCCTGCTTAATGAGGCAGAGAAAAGTT
GACTTGCCTAGTTTGAATTGGCTGGGTGGCTTTTCGTCAAGGAGTGGAATGCGGATTTCAAGGACG
AACTAATAAATTGGTTGATGGTTGCTACTCCTTTTGGCAGGGAGCTGCCATTGCTTTCACACAAA
AGTTAATTACGATTGTTGATAAGCAATTGAGGTCTCGTATTCTGCAAAAGGCCATCAGGAGAG
GATGCCTGCAGCACCAGTTCATATGGGTGCACCGCAATAAGTCTTCTCTGCTGTGGACTATGC
GAAGTTTGGATTTGATTTTATACAACAGAGCAACCAATTGGCCCACTCTCCATAACATTGCCC
TGCAACAATACATCCTACTTTGTTCTCAGGTAAGAGGGAGGCTTGAGGGATAAGCCTGGAAAG
AACAGAGATCACTATCATTGATGCTACTGCCTCAGTGGCCTCGCAGTTAGCCAGTACAGTGCCAT
GACTGATACTGGTTCTGTCGCCATTACCTCAGCATGTGCTTGGACCGTACTCTAATTTGCTGGAGC
CAATCCATCC

```

A disclosed FT6 polypeptide (SEQ ID NO:45) encoded by SEQ ID NO:43 has 414 amino acid residues and is presented in Table 9B using the one-letter amino acid code.

**Table 9B. Encoded FT6 protein sequence (SEQ ID NO:45).**

```

ADPDLPRLTVTQVEQMKVEARVGD IYRSLFGAAPNTKSIMLELWRDQHI EYLTP
GLRHMGP AFHVL DANRPWLCYWMVHPLALLDEALDDDLENDI IDFLARCQDKDG
GYSGGPGQLPHLATTYAAVNTLVTIGSERALSSINRGNLYNFM LQMKDVSGAFR
MHDGGEIDVRASYTAISVASLVNILD FKLAKGVGDYIARCQTYEGGIAGEPYAE
AHGGYTFCGLAALILLNEAEKVDLPSLIGWVAFRQGV ECGFQGR TNKLV DGCYS
FWQGAAIAFTQKLITIVDKQLRSSYSCKRPSGEDACSTSSYGCTANKSSSAVDY
AKFGFDFIQSNQIGPLFHNIALQQYILLCSQVLEGG LRD KPGKNRDHYHSCYC
LSGLAVSQYSAMTD TGSCPLPQHVLGPYSNLL EPIH

```

Due to the nature of the cloning strategy the sequence presented is not full length. The percent identity of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other sequences is shown in Figure 17.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

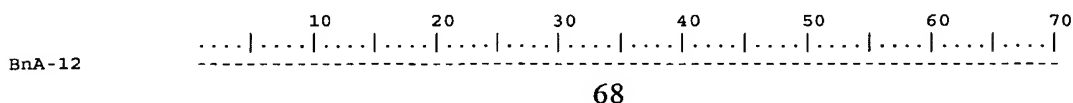
The present invention also includes a nucleic acid sequence complimentary to the *Zea mays* beta subunit of SEQ ID NO:43. The disclosed complementary sequence is shown as SEQ ID NO:44.

SEQ ID NO:44

The FTA and FTB nucleic acids and amino acids disclosed above have homology to other members of the FT protein family (GenBank ID NOs: U63298, U83707, and U73203; WO 00/14207; Cutler et al., Science 273(5279):1239-41, 1996; Ziegelhoffer et al., Proc Natl Acad Sci U S A. 97(13):7633-8, 2000). The homology between these and other sequences is shown graphically in the ClustalW analysis shown in Tables 10A-10D. In the ClustalW alignment, the black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or functional properties), whereas non-highlighted amino acid residues are less conserved and can potentially be altered to a much broader extent without altering protein structure or function.

**Table 10A. ClustalW Nucleic Acid Analysis of FT Alpha Subunits**

- 1) BNA-12; FT2 (SEQ ID NO:12)
- 2) At-FT-A; FT1 (SEQ ID NO:7)
- 3) PPI-Soy-FTA; FT4 (SEQ ID NO:37)
- 4) Pea-FT-A (SEQ ID NO:65)
- 5) Tomato-FTA (SEQ ID NO:66)
- 6) Rice-FT-A (SEQ ID NO:67)
- 7) Zea mays-FT-A (SEQ ID NO:68)
- 8) Soy1-FT-A (SEQ ID NO:69)
- 9) Soy2-FT-A (SEQ ID NO:70)
- 10) Triticum-FT-A (SEQ ID NO:71)



69

BnA-12	CAGTTGTGG-----	CATCATCCACGATGGGTCGCGAGAGA
At-FT-A	CAACTGTGG-----	CATCATCCGCGATGGGTTGCAGAGA
PPI-Soy-FTA	CAGATGTGN-----ATGTTCTG-----TAG-----	CATCCTAGACGATGGGTTGCCGAGA
Pea-FT-A	CAGATTTGG-----	CATCATAGACGATGGGTTGCTGAGA
Tomato-FTA	CAAAATATGG-----	CATCATAGACGCTGGCTTGCTGAGA
Rice-FT-A	CAAAATCTGG-----	CATCAAAAGAGATGGCTTGCGGAGA
Zea mays-FT-A	CAAAATCTGG-----	CACATAAGAGATGGCTTGCTGAGA
Soy1-FT-A	CAGATGTGG-----	CATCATAGACGATGGGTTGCCGAGA
Soy2-FT-A	CAGATGTGGTGATGCTCTGCTCTGCTCTTCTTCCATACTTTG	CATCATAGACGATGGGTTGCCGAGA
Triticum-FT-A	CAACTCTGG-----	CATCAAAAGAGATGGCTTGCTGAGA
	500 510 520 530 540 550 560	
BnA-12	AACTGGGTCCTGATGTTGCAAGGAAAGGAACCTTGAGTTTACTCGGAGCTACTATCAGTTGATGCCAAGCA	
At-FT-A	AACTGGGTCCTGATGTTGCAAGGAGAGAACTTGAAATTTACCCGTAGAGTACTTTCACTTTGATGCCAAGCA	
PPI-Soy-FTA	AGTTAGGTCCTGAAGCTAGAAACAATGAGCTCGAGTTCAACAAAAGATACTGTCCCTTGATGCCAAGCA	
Pea-FT-A	AATTAGGACCTGAAGCTAGAAACAATGAGCTTGAGTTCAACAAAAGATTTCTGTCTGTGACGCCAAGCA	
Tomato-FTA	AGCTGGAGCTGATGCTGTGCAAAATGAGCTAGAATTCACCAAGAAATATTTCTCAGGATGCCAAGCA	
Rice-FT-A	AATTAGGACCAATATTTGCAAAATAGAGACCAATTTACAAGGAAGATACTTTCTATGATGCCAAGCA	
Zea mays-FT-A	AATTAGGACCTGCTATTTGCAAAACAAGAGCATGAATTCACAAATGAAGATACTTTGCTATTTGATGCCAAGCA	
Soy1-FT-A	AGTTAGGTCCTGAAGCTAGAAACAATGAGCTCGAGTTCAACAAAAGATACTGTCCCTTGATGCCAAGCA	
Soy2-FT-A	AGTTAGGTCCTGAAGCTAGAAACAATGAGCTCGAGTTCAACAAAAGATACTGTCCCTTGATGCCAAGCA	
Triticum-FT-A	AATTAGGACCAATGATGCTGCAAAATGAGCATGAGTTCAAAAGGAAGATACTTTGCTATTTGATGCCAAGCA	
	570 580 590 600 610 620 630	
BnA-12	TTATCATGCTTGGTCAATAGGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAGATGAACCTTAACCTAC	
At-FT-A	TTATCATGCTTGGTCAATAGGCAGTGGGCACTACGGGCAATTAGGAGGATGGGAAGATGAACCTTAACCTAC	
PPI-Soy-FTA	TTATCATGCTTGGTCTCATAGACAGTGGGCTCTTCAAAACACTAGGAGGATGGGAAGATGAACCTTAACCTAC	
Pea-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCTCTTCAAAATCTAGGAGGATGGGAAGATGAACCTTAACCTAC	
Tomato-FTA	TTATCATGCTTGGTCTCATAGGCAGTGGGCTCTTCAAGCACTTGGAGGATGGGAAGATGAACCTTAACCTAC	
Rice-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCTCTTCAAGCACTTGGTGGATGGGAAGATGAACCTTAACCTAC	
Zea mays-FT-A	TTATCATGCTTGGTCTCATAGGCAGTGGGCTCTTCAAGCCTTGGGCGATGGGAAGATGAACCTTAACCTAC	
Soy1-FT-A	TTATCATGCTTGGTCTCATAGACAGTGGGCTCTTCAAAACACTAGGAGGATGGGAAGATGAACCTTAACCTAC	
Soy2-FT-A	TTATCATGCTTGGTCTCATAGACAGTGGGCTCTTCAAAACACTAGGAGGATGGGAAGATGAACCTTAACCTAC	
Triticum-FT-A	CTACCATGCTTGGTCTCATAGGCAGTGGGCTCTTCAAGCATTGGGCGATGGGAAGATGAACCTTAACCTAC	
	640 650 660 670 680 690 700	
BnA-12	TGCAACGAGCTCCTTGAAGCTGACCTCTTTAAACAATCTGCAATGGAATCAGAGGATATTACCTTATAACCTA	
At-FT-A	TGCAACGAGCTCCTTGAAGCTGACCTCTTTAAACAATCTGCAATGGAATCAGAGGATATTATGTCATCAACCTA	
PPI-Soy-FTA	TGCAACGAGCTCCTTGAAGAGACATTTTAAACAATCTGCTTGAATCAGAGATATTGTCATAACCTA	
Pea-FT-A	TGCAACGAGCTCCTTGAAGAGACATTTTAAACAATCTGCTTGAATCAGAGATATTGTCATAACCTA	
Tomato-FTA	TGCAACGAGCTCCTTGAAGAGATATTTAAACAATCTGCTTGAATCAGAGATATTGTCATAACCTA	
Rice-FT-A	TGCAACGAGCTCCTTGAAGAGAGCTCTTCAAAATTCAGCTTGAATCAGAGATACCTTGTATAACCTA	
Zea mays-FT-A	TGCAACGAGCTCCTTGAAGAGAGCTCTTCAAAATTCAGCTTGAATCAGAGATATTGTCATAACCTA	
Soy1-FT-A	TGCAACGAGCTCCTTGAAGAGAGATTTTAAACAATCTGCTTGAATCAGAGATATTGTCATAACCTA	
Soy2-FT-A	TGCAACGAGCTCCTTGAAGAGAGATTTTAAACAATCTGCTTGAATCAGAGATATTGTCATAACCTA	
Triticum-FT-A	TGCAACGAGCTCCTTGAAGAGAGATCTTCAAAATTCAGCTTGAATCAGAGATACCTTGTGTAACCTA	
	710 720 730 740 750 760 770	
BnA-12	GATCACCCTTCTGTTGGCAGGCTAGAAAGCATGAGAGAACTCTGAAGTAAGCTACACAGTCAAAGCCATTAT	
At-FT-A	AATCTCCTTTCTTGGCAGGCTAGAAAGCATGAGAGAACTCTGAAGTAAGCTACACAGTCAAAGCCATTAT	
PPI-Soy-FTA	GGTCTCCTTTCTTGGGCGGCTAAAGCATGAGAGAACTCTGAAGTCTTTACACAGTCAAAGCCATTAT	
Pea-FT-A	GGTCTCCTTTCTTGGGCGGCTAAAGCATGAGAGAACTCTGAAGTCTTTTACAGCTTGAAGCCATTAT	
Tomato-FTA	GATCACCCTCTACGAGGCGCTAGTGGCAATGAGCGAATTTGAAGTGAATTACACAGTTCAAAGCCATTAT	
Rice-FT-A	GTTCAACCACTTCTTGGAGGCTTGCAGCAATGCTGACTCGGAAGTGAATTACACAGTTGGGCTATTCT	
Zea mays-FT-A	GATCACCCTTCTTGGTGGCCTTGCAGCAATGCTGATTCAAGAGTGAATTACACAGTTGAAGCTATTCT	
Soy1-FT-A	GGTCTCCTTTCTTGGGCGGCTAAAGCATGAGAGAACTCTGAAGTCTTTACACAGTTGAAGCCATTAT	
Soy2-FT-A	GGTCTCCTTTCTTGGGCGGCTAAAGCATGAGAGAACTCTGAAGTCTTTACACAGTTGAAGCCATTAT	
Triticum-FT-A	GATCACCCTTCTTGGGCGGCTTGCAGCAATGCTGACTCAAGAGTGAATTACACAGTTGAAGCCATTAT	
	780 790 800 810 820 830 840	
BnA-12	AGCAAAATCCCGGAAAGAGAGCTCTTGGAGCTACCTGAAAGCCCTTTACAAAGACGACACAGAGCTCTTGG	
At-FT-A	AGCAAAATCCCGCAAAAGAGAGCTCTATGGCGATACCTAAAGCCCTTTACAAAGACGACAAAGAACTCTTGG	
PPI-Soy-FTA	AGCCTACCCCTGAAATGAAAGCTCTGGAGATATCTACGAGGACTTTTAAAGGTGAAAGTACTTCAATGG	
Pea-FT-A	TTCTTACCCAGAAATGAAAGCTCTGGAGATATCTTGGAGGACTTTTCAAGATGAATCAACCTTCAATGG	
Tomato-FTA	AGCTTAGTCCAGACAATGAAAGCTCTGGAGATATCTTCTGCTCTTACAAAGATGATACACAATCTCTA	
Rice-FT-A	GGCTAACCCCTCAGAAATGAAAGCCCTGGAGATACCTCAAAGCCCTGTACAAAGGTGAAATAACTTGTCTG	
Zea mays-FT-A	AGCAAAATCCCTCAGAAATGAAAGCCCTGGAGATACCTCAAAGGCTCTATACAAAGGTGAAATAACTTGTCTG	
Soy1-FT-A	AGCCTACCCCTGAAATGAAAGCTCTGGAGATATCTACGAGGACTTTTAAAGGTGAAAGTACTTCAATGG	
Soy2-FT-A	AGCCTACCCCTGAAATGAAAGCTCTGGAGATATCTACGAGGACTTTTAAAGGTGAAAGTACTTCAATGG	
Triticum-FT-A	GTTGAACCCCTCAGAAATGAAAGCTCTGGAGATACCTCAGAGCTTTTATTAAGATGATACAAATTTGTCTG	
	850 860 870 880 890 900 910	

BnA-12	ATTTAGTGATCCAAAGTGTTCCTCAGTCTGTTTGAAGTTCTCTACCGCGCGGACTGTTCCATGGATTCC
At-FT-A	ATTAGTGATCCAAAGTGTTCCTCAGTCTGTTTGAAGTTCTATCCCGCACAGATTGTTCCATGGATTCC
PPI-Soy-FTA	GTAAATGATCCCAAGTATCTTCAATATGTTTAAAGATTTTGAGAACTAAGAGCAAC---TACCTGTTTG
Pea-FT-A	GTAAATGATCCCAAGTATCTTCAATATGTTTAAAGATTTTGAGAACTAAGAGCAAC---TATTTGTTTG
Tomato-FTA	GTTCAGSATTCGAAGTAGCATCAGTACTTTGGGACGCTTAACTCCCAAAATAGT---CATGTGCACG
Rice-FT-A	ATGGTGATGAGCGCATCTCTGATSTTTGTCTCAAGGTCCTGAAACATGATTCCAGC---TGCCTATTTG
Zea mays-FT-A	GTAGAGGACGAGCGCATCTCTCTCTTTGTTTCAAGGTCCTGAAGAAATGATTGGACT---TGTGTATTTG
Soy1-FT-A	GTAAATGATCCCAAGTGTTCCTCAGTATGCTTAAAGATTTTGAGAACTAAGAGCAAC---TACCTGTTTG
Soy2-FT-A	GTAAATGATCCCAAGTGTTCCTCAGTATGCTTAAAGATTTTGAGAACTAAGAGCAAC---TACCTGTTTG
Triticum-FT-A	GTGGCTGATAATGGCAATTTCTGATCTTGCTCAAGGTCCTGAATAAGGATTGGACA---TGCCTATTTG
920 930 940 950 960 970 980	
BnA-12	CTCTGAGCACCTTTTGGATCTTCTGTGGATGGCT-TGAGACCAACCAACGAGCATAGAGACTCGGTGA
At-FT-A	CTCTGAGCACCTTTTGGATCTTCTATGTGATGGAC-TGAGACCAACCAACGAGCATAAAGACTAGTGA
PPI-Soy-FTA	CTCTTAGCACTATTTTAGATCTTATATGCTTTGGTTAT-CAACCAAATGAAGACATTAGAGATGCCATTG
Pea-FT-A	CTCTTAGCACTATTTTAGATCTTATATGCTTTGGTTAT-CAACCAAATGAAGACATTAGAGATGCCATTG
Tomato-FTA	CTCTAGTACTCTGCTCGATCT-ATCTGCTCTGCTTATCAACCAAATGAAGATTTCAGAGATGCCATTG
Rice-FT-A	CTCTGAGTTCTTGTGGATCTTCTTTGCTCATGATT-TGGAACCGAGCCAAAGAAATGAAAAGTGGCTGAG
Zea mays-FT-A	CTTTGAGCTTGTGCTCGATCTTCTTCAAATTTGGTT-TACAACCTTCAGATGAACCTCAAAGCAATATCG
Soy1-FT-A	CTTTGAGCTTGTGCTCGATCTTCTTCAAATTTGGTT-TGCAACCTTCAGATGAACCTTAGGTCCACTTG
Soy2-FT-A	CTTTGAGCTTGTGCTCGATCTTCTTCAAATTTGGTT-TGCAACCTTCAGATGAACCTTAGGTCCACTTG
Triticum-FT-A	CTTTGAGCTTGTGCTCGATCTTCTTCAAATTTGGTT-TGCAACCTTCGAATGAACCTTAAAGCAACATCG
990 1000 1010 1020 1030 1040 1050	
BnA-12	AAGCTCTAG---CT---AATGAAGA---A---CCAGAGAC
At-FT-A	GAGCTCTAG---CT---AATGAAGA---A---CCAGAGAC
PPI-Soy-FTA	ACGCCTTAAAGACCGCAGATATGGATAACAAGATTTAGATGATGATGAGAAAGGGGAACAACAAATTT
Pea-FT-A	AGGCTTTAA-GACTTCAGATTTTGATAAA---A---CAAGATTC
Tomato-FTA	ATGTTCTTA---CTCCC---CACTCAGCTC---A---CCAGATTG
Rice-FT-A	AAACAAATAAGAACTCTGATCCTGAAGCAGATGA---AG---CA-GTAGATGC
Zea mays-FT-A	AAACAAATAAGAACTCTGATCCTGAAGACCGC---G---GATGA
Soy1-FT-A	ACGCCTTAAAGACCGCAGATATGGATAACAAGATTTAGATGATGATGAGAAAGGGGAACAACAAATTT
Soy2-FT-A	ACGCCTTAAAGACCGCAGATATGGATAACAAGATTTAGATGATGATGAGAAAGGGGAACAACAAATTT
Triticum-FT-A	AAGCAATGGAGAACTCTGATCCTGAAGACGGG---A---ACATGC
1060 1070 1080 1090 1100 1110 1120	
BnA-12	TAACCTTGGCAATTTGGTCTGTACCATTTCTGTGCTGTGTGATCCAATAAGAGCTAACTATTGGGCATGG
At-FT-A	TAACCTTGGCAATTTGGTCTGTACTATTCTTGGTCTGTGATCCCTATAAGAGCTAACTATTGGGCATGG
PPI-Soy-FTA	AAATATAGCAGCAAAATATTTGTTCTATCCCTAAACAAGTTGATCCAATTAGAACCAACTATTGGATTGG
Pea-FT-A	AGATATAGCAATAACTATTTGTTCTATTTTGAACAAGTTGATCCAATTAGAGTCACTATTGGGCTCTGG
Tomato-FTA	AGCACTGACAAAGAAATTTGTTCCATCTTGGAAACATCTGATCCAATGAGAGTAAATATTGGAAATGG
Rice-FT-A	TGATCTTGGCACTGCAATCTGCTCAATATTCGAGAGATGTGATCCCTGCGCGATAAATTACTGGTCTCTGG
Zea mays-FT-A	TGATCTTGGCACTGCAATCTGCTCAATATTCGAGAGATGTGATCCCTGCGCGTAAATATTGGTCTCTGG
Soy1-FT-A	AAATATAGCAGCAAAATATTTGTTCTATCCCTAAACAAGTTGATCCAATTAGAACCAACTATTGGATTGG
Soy2-FT-A	AAATATAGCAGCAAAATATTTGTTCTATCCCTAAACAAGTTGATCCAATTAGAACCAACTATTGGATTGG
Triticum-FT-A	TGATATTGCACTAGCTCTCTGCTCAATCTCTGAGAAATGTGATCCCTGCGCGATAAATACTGCTCATGG
1130 1140 1150 1160 1170 1180 1190	
BnA-12	AGGAAGAGCAAGATTACA---GTGGC-AGCAATTTGAATATGTGACGCCCCAAATCACACTTGAAAAA
At-FT-A	CGCAAGAGCAGACTTC---CT---CTCA-GGCAGCTAAAGGCAAACTTATGTCATATGTGTAATTTTA
PPI-Soy-FTA	CGCAAGAGCAGACTTC---CT---CTCA-GGCAGCTAAAGGCAAACTTATGTCATATGTGTAATTTTA
Pea-FT-A	CGCAAGAGCAGACTTC---CT---CTCA-GGCAGCTAAAGGCAAACTTATGTCATATGTGTAATTTTA
Tomato-FTA	TACAGGACCACTATTCT---TCTCA-AAC---CTGAAG---CATGCAGTGGCCTCCATCA---GG
Rice-FT-A	TTCAAGGACACTCTTTCTCAGATCTCATGAGCTTACATGGGTTCACCCCTTGTCCGCGCTGTCGGGGCT
Zea mays-FT-A	CGCAAGAGCAGACTTC---CTCT-ATCAGCTTAGTAACCAAGTAATTAA---GGCAACTCTGT
Soy1-FT-A	CGCAAGAGCAGACTTC---CTCT-ATCAGCTTAGTAACCAAGTAATTAA---GGCAACTCTGT
Soy2-FT-A	CGCAAGAGCAGACTTC---CTCT-ATCAGCTTAGTAACCAAGTAATTAA---GGCAACTCTGT
Triticum-FT-A	TACAGAGCACTCTTTCT---TCTTA-GACATCTGAAA-TTCAGCTGAAGACAGTTTTAG---CA
1200 1210 1220 1230 1240 1250 1260	
BnA-12	GACTTGATTAT---TAGT-TTTTACGT-----AATTAACGCTTATTATGAATCACA-TTTCAT
At-FT-A	GACTTGATTAT---TAGT-TTTTACGT-----AATTAACGCTTATTATGAATCACA-TTTCAT
PPI-Soy-FTA	GTCTATTGGAATTTGACGTCATGGAT-----AACAGGGTGGTTGTTTGTGTTATGATAT-GTTTT
Pea-FT-A	GTCTATTGGAATTTGACGTCATGGAT-----AACAGGGTGGTTGTTTGTGTTATGATAT-GTTTT
Tomato-FTA	GTCTATTGGAATTTGACGTCATGGAT-----AACAGGGTGGTTGTTTGTGTTATGATAT-GTTTT
Rice-FT-A	TCATTAATGGAGATATCTTCTAT-----CTTCGTTGTA-----TTCTG
Zea mays-FT-A	CTCTGAGATAGACATGTTTATGATAGTTTCATTGGACACCCAAACAGAGCGGACAGAGTGATGGCTGCT
Soy1-FT-A	GTCTATTGGAATTTGACGTCATGGAT-----AACAGGGTGGTTGTTTGTGTTATGATAT-GTTTT
Soy2-FT-A	GTCTATTGGAATTTGACGTCATGGAT-----AACAGGGTGGTTGTTTGTGTTATGATAT-GTTTT
Triticum-FT-A	GCAATGATGTAACTCAATCGAAGGGGTT-----GACGCAGCTGATGAAAAACCTTTTCCT

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      1270      1280      1290      1300      1310      1320      1330
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
BnA-12
At-FT-A  GT-TAACATGTTATCAAAACAATCTTGATTCTCAAAAAAAAAAAAAAAAAAAAAA-----
PPI-Soy-FTA
Pea-FT-A  CC-AGATGTATTTCCTATATTAAACAGCAAAGTTGATTTAACATGGTGTTAAACAACTGATCTCCAA
Tomato-FTA ATCTAAGGTGATCCTTCGGGCACATGTGCTGGGAAGTGACTGAATATCACCAGAACTAAAAAACTGTG
Rice-FT-A  GGCGTTGAGGTGCCT---ACCTACATTGTTATGAACCTTCCTGGGCATAACTGATCACTGATATTAC
Zea mays-FT-A ACCTTCTCCGTGACTGAAAGCAGTGCTTGTACCA--TTTGTTTAGTAAATTTGTGAGTGTACTGCT
Soy1-FT-A  GT-TGTCATGTATCTGTTGT---GCAAATTT-----ATCTTTTGTGTCATGCCATTACTGGCATTGGA
Soy2-FT-A  GT-TGTCATGTATCTGTTGT---GCAAATTT-----ATCTTTTGTGTCATGCCATTACTGGCATTGGA
Triticum-FT-A GTGATCTTGGTSCGG--AGCAA--TTTGTACTCA--TTTACTGGGAAAAATCAATCAATGACAGCATG

      1340      1350      1360      1370      1380      1390      1400
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A  AAAATCAATGTTTATTCTCTTCATTGTTCTGATTTTGTGGCATAACATTCTTGATGAT-TTTGTGGTA
Tomato-FTA ATTGGCAACATTGTACTACTCCAAATAGGTCACCTTCGATGACTTTTGTACTGCCTTGA-GTTTTGGCT
Rice-FT-A  TCCAATATGTGTTCTAAA-----
Zea mays-FT-A CCAAACAACACCTTATGCAACCATATTGGAATAT---TTCACATGTAAGCT---TGA-----A-TC
Soy1-FT-A  GTG--TAAGGATTGAAAGCCATGCA-----GAATAAGAAATTAAGTTTTTT-----TTTCCGTTG
Soy2-FT-A  GTG--TAAGGATTGAAAGCCATGCA-----GAATAAGAAATTAAGTTTTTT-----TTTCCGTTG
Triticum-FT-A CCCAACAATGTCTTGTGTGAATATGTTACTGCCTGATATTCACATGTTAGCAGAATGAGAAATAACCAATC

      1410      1420      1430      1440      1450      1460      1470
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A  AAAAAAAAAAAAAAAAAAAAAA-----
Tomato-FTA CTGCTATGTTTTGTAAAGTTTTGGATATGGATGCATAGCTTATTGATACTTTTGGTGACTTAAATACTCT
Rice-FT-A
Zea mays-FT-A CAGGTGTGTTTGTTAATGTATTACACTT--G-CCATGGGAGCCTAAATGAGACCCATAATCACTTCCACT
Soy1-FT-A  AAAA-----
Soy2-FT-A  AAAAAAAAAAAAAAAAAAAAAA-----
Triticum-FT-A AAACCTCAACGAGCAGATTGTTACAGTAACGGCCACTGGTGGTGTGAAAATCCTGAAATCTGCTTCAGTC

      1480      1490      1500      1510      1520      1530      1540
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A
Tomato-FTA GGAAGGCAGGTAGCATGTGTATAATTCAGTGTTACTTCCCATGTCGAGTTAGATGCTTGAAAAATTTAGT
Rice-FT-A
Zea mays-FT-A AGAGTCGGAAGACCGT-GTCGAGCAGTTCACTCATATGGTCACTTAAAGCAAAAAAAAAAAAAAAAAA--
Soy1-FT-A
Soy2-FT-A
Triticum-FT-A ACTTTGCCTTGTTTACAGTTGAGTCTGTTGTTGTGATCTGTACCTAATGCATGTACACAATCATCAAATT

      1550      1560      1570      1580      1590      1600      1610
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
BnA-12
At-FT-A
PPI-Soy-FTA
Pea-FT-A
Tomato-FTA AGGTGTTCTTTTATGAAGCACACATTAATGTGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Rice-FT-A
Zea mays-FT-A
Soy1-FT-A
Soy2-FT-A
Triticum-FT-A ATTAGTTTTGTACCAATGAGTATTCGATGAAAAAAAAAAAAAAAAA-----

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BnA-12      -
At-FT-A     -
PPI-Soy-FTA -
Pea-FT-A    -
Tomato-FTA  A
Rice-FT-A   -
Zea mays-FT-A -
Soy1-FT-A   -
Soy2-FT-A   -
Triticum-FT-A -

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Table 10B. ClustalW Amino Acid Analysis of FT Alpha Subunits

- 1) BnA-12; FT2 (SEQ ID NO:13)
- 2) At-FT-A; FT1 (SEQ ID NO:11)
- 3) PPI-Soy-FTA; FT4 (SEQ ID NO:39)
- 4) Pea-FT-A (SEQ ID NO:72)
- 5) Tomato-FTA (SEQ ID NO:73)
- 6) Rice-FT-A (SEQ ID NO:74)
- 7) Zea mays-FT-A (SEQ ID NO:75)
- 8) Soy1-FT-A (SEQ ID NO:76)
- 9) Soy2-FT-A (SEQ ID NO:77)
- 10) Triticum-FT-A (SEQ ID NO:78)

	10	20	30	40	50	60	70
BnA-12	..... ..... ..... ..... ..... ..... ..... .....	MDYFRAIYFSDESRARLR					
At-FT-A	-----MNFDET	YPLSQRL	EWSDVVP	ETQDDGP	PVVPI	AYKEEFRE	MDYFRAIYFSDESRARLR
PPI-Soy-FTA	-MESGSSEGE	EVQQRV	PLRER	VEWSDVT	PVPQNDGPN	PVVPIQY	TEEFSEVMDYFRAVYLDERSPRALA
Pea-FT-A	--MAGNIEVEE	-DDR	VPLRL	FEWSDVT	P	PODDGPS	PVVPIQYSEEFSEVMDYFRAVYFAKELSSRALA
Tomato-FT-A	-----MDSCEVT	KTRIP	FKER	PDWADV	KVPVQDDGP	CPVVPIAY	TEDFSEMDYFRAIYVADERSRALQ
RiceFT-A	MAPSSSTSE	GASDEW	PPSR	RPPL	ADVV	PVPTQDDGPH	PVVVAIAYRDEEFREVMYFRAIYFAGERSVRALH
Zea mays-FT-A	-----MEHTLSG	PSSWPEL	ADVV	PVPQDDGPS	PVVSI	AYRDDFRG	VMDYFRAIYLTGERSPRALR
Soy1-FT-A	-MESGSSEGE	EVQQRV	PLRER	VEWSDVT	PVPQNDGPN	PVVPIQY	TEEFSEVMDYFRAVYLDERSPRALA
Soy2-FT-A	-MESGSSEGE	EVQQRV	PLRER	VEWSDVT	PVPQNDGPN	PVVPIQY	TEEFSEVMDYFRAVYLDERSPRALA
Triticum-FT-A	-----DVA	ETPQ	ADG	PCPVV	SIAYR	GEF	REVMYFRAIYFAGERSPRALR

	80	90	100	110	120	130	140
BnA-12	LTBEA	RLNSG	NYTVWH	FRLLE	ELN	DLYEEL	KFIESIAEDNSKNYQIW
At-FT-A	LTBE	TLN	SGNYTVWH	FRRLL	LEAL	UNHDL	FELEFIERIAEDNSKNYQIW
PPI-Soy-FTA	LTA	EAVQ	RNSGNYTVWH	FRRLL	LES	LKVDL	NDELEFVERMAAGNSKNYQIX
Pea-FT-A	LTA	EAL	CLNAGNYTVWH	FRRLL	LES	LKVDL	HVEREFVERMAAGNSKNYQIW
Tomato-FT-A	LT	GEAL	CLNPGNYTVW	QFRRV	LEAL	GVDL	REELKFVERIAGENTKNYQIW
RiceFT-A	LTA	EV	CLNPGNYTVWH	FRRLL	LEAL	DADL	REEMDFVERIVECNPKNYQIW
Zea mays-FT-A	LTA	EAL	CLNPGNYTVWH	FRRLL	LES	LDFOL	LEEMKFVELIAECNPKNYQIW
Soy1-FT-A	LTA	EAVQ	RNSGNYTVWH	FRRLL	LES	LKVDL	NDELEFVERMAAGNSKNYQIW
Soy2-FT-A	LTA	EAVQ	RNSGNYTVWH	FRRLL	LES	LKVDL	NDELEFVERMAAGNSKNYQIW
Triticum-FT-A	LTA	EAL	CLNPGNYTVWH	FRRV	LEAL	DADL	LLEMFHVDGIAESNPLNYQIW

	150	160	170	180	190	200	210
BnA-12	EKLG	PDVAGL	EKEFT	FRVLS	DAKH	YAWSHRQW	ALQALGGWENELNYCHELLEADYFNNSAWNORYYVI
At-FT-A	EKLG	PDVAGL	EKEFT	FRVLS	DAKH	YAWSHRQW	TLRALGGWEDELNYCHELLEADYFNNSAWNORYYVI
PPI-Soy-FTA	EKLG	PEAR	NNELEFT	KKIL	SDAK	HYAWSHRQW	ALQALGGWEDELNYCTELLKEDIFNNSAWNORYFVI
Pea-FT-A	EKLG	PEAR	NNELEFT	KKIL	SDAK	HYAWSHRQW	VLCNLGGWEDELNYCSELLAEDIFNNSAWNORYFVI
Tomato-FT-A	EKLG	ADAV	TNELEFT	KKIL	SDAK	HYAWSHRQW	VLCNLGGWEDELNYCQELLEDFNNSAWNORYFVI
RiceFT-A	EKLG	PDI	ANKEHEFT	KKIL	SDAK	HYAWSHRQW	VLCNLGGWETELQYCNQELLEDFNNSAWNORYYVI
Zea mays-FT-A	EKLG	PDI	ANKEHEFT	KKIL	SDAK	HYAWSHRQW	VLCNLGGWETELQYCDHLLKEDYFNNSAWNORYFVI
Soy1-FT-A	EKLG	PEAR	NNELEFT	KKIL	SDAK	HYAWSHRQW	ALQALGGWEDELNYCTELLKEDIFNNSAWNORYFVI
Soy2-FT-A	EKLG	PEAR	NNELEFT	KKIL	SDAK	HYAWSHRQW	ALQALGGWEDELNYCTELLKEDIFNNSAWNORYFVI
Triticum-FT-A	EKLG	PDI	ANSEHEFT	KKIL	SDAK	HYAWSHRQW	VLCNLGGWSELOQYCNQELLEDFNNSAWNORYYVI

	220	230	240	250	260	270	280
BnA-12	TRSP	SLGGL	EAMRESEV	SYTVA	IAE	IANP	NESSWRYLKALYKDBESWISDPSVSSVCLKVL
At-FT-A	TRSP	SLGGL	EAMRESEV	SYTVA	IAE	IANP	NESSWRYLKALYKDBESWISDPSVSSVCLNVL
PPI-Soy-FTA	TRSP	FLGGL	KAMRESEV	LYT	HEA	IAYP	NESSWRYLRGLYKCEITISWVNDPQVSSVCLKIL
Pea-FT-A	TRSP	VLGGL	KAMRESEV	LYT	HEA	IAYP	NESSWRYLRGLYKCEITISWVNDPQVSSVCLKIL
Tomato-FT-A	TRSP	LLGGL	VAMRELEV	NYT	GAIR	SPENES	PWRYLRGLYKNDTOSLVODSEVASVLWDVL
RiceFT-A	TRSP	LLGGL	AAMRDS	EV	DTY	CAIR	ANPONESPWRYLRGLYKCEITISWVNDPQVSSVCLKIL
Zea mays-FT-A	TRSP	FLGGL	AAMRDS	EV	DTY	HEA	IAYPNESSWRYLRGLYKCEITISWVNDPQVSSVCLKIL
Soy1-FT-A	TRSP	FLGGL	KAMRESEV	LYT	HEA	IAYP	NESSWRYLRGLYKCEITISWVNDPQVSSVCLKIL
Soy2-FT-A	TRSP	FLGGL	KAMRESEV	LYT	HEA	IAYP	NESSWRYLRGLYKCEITISWVNDPQVSSVCLKIL
Triticum-FT-A	TRSP	ILGGL	AAMRDS	EV	DTY	HEA	IAYPONESPWRYLRGLYKCEITISWVNDPQVSSVCLKIL

	290	300	310	320	330	340	350
BnA-12	FAL	STLLD	LLC	GLR	P	TNE	HRDSVKALANE
							ETN
							LANL
							CTAT
							CEVD
							PIRAN

At-FT-A	FALSTLLDLLCDGLRPINSHKDSVRALANEPP---ETN-----LANLAVCTILGRVDPIRAN
PPI-Soy-FTA	FALSTLLDLLICFCYQPNEDIRDAIBALKTAEM---DKQDLDDDEKGEQQNLNTARNICCSILKQVDPIRIN
Pea-FT-A	FALSTLLDLSASVHQPNEDERDAIBALKLQIL---IKQ--DSD-----LAITICSILEQVDPIRVN
Tomato-FT-A	HALRFLLDLLCHDLEPSOELKSAVEVLTPQSC---SPD-----LAITKKICSILEHADPMRVK
RiceFT-A	FALSLLDLLLQIGLOPSDELKGTIEATKNSEPEADBAVDA--D-----PATAICSILOKCDPIRIN
Zea mays-FT-A	FALSLLDLLLCTGLQPSGGRSTEGTIRSSHP---ETADD--L-----PAAAVCCILOKCDPLAVN
Soy1-FT-A	FALSTLLDLLICFCYQPNEDIRDAIBALKTAEM---DKQDLDDDEKGEQQNLNTARNICCSILKQVDPIRIN
Soy2-FT-A	FALSTLLDLLICFCYQPNEDIRDAIBALKTAEM---DKQDLDDDEKGEQQNLNTARNICCSILKQVDPIRIN
Triticum-FT-A	FALSFLDLLLRMGLOPSNELKGTIEAMENSEP---ETGHA--D-----PAVAVCSILOKCDPIRIN

	360	370
BnA-12	YVAVKLL	-----
At-FT-A	YVAVRKSRLTVAAI	-----
PPI-Soy-FTA	YVAVRKSRLP	-----
Pea-FT-A	YVAVRKSRLPQAA	-----
Tomato-FT-A	YVAVRKSRLRVQLLSQNAERLANLSVQE	-----
RiceFT-A	YVAVRKSRLTSSQT	-----
Zea mays-FT-A	YVAVRKSRLTSSQIS	-----
Soy1-FT-A	YVAVRKSRLPLSA	-----
Soy2-FT-A	YVAVRKSRLPLSA	-----
Triticum-FT-A	YVAVRKSRLTSS	-----

Table 10C. ClustalW Nucleic Acid Analysis of FT Beta Subunits

- 1) PPI-BnFTb; FT3 (SEQ ID NO:14)
- 2) eral (SEQ ID NO:1)
- 3) Wiggum (SEQ ID NO:80)
- 4) PPI-Soy-FTB; FT5 (SEQ ID NO:40)
- 5) DuP-Soy-FTB (SEQ ID NO:81)
- 6) PPI-Corn-FTB; FT6 (SEQ ID NO:43)
- 7) DuP-Corn-FTB (SEQ ID NO:82)
- 8) Pea-FT-B (SEQ ID NO:83)
- 9) Tomato (SEQ ID NO:84)
- 10) Tobacco (SEQ ID NO:85)

	10	20	30	40	50	60	70
PPI-BnFTb	..... ..... ..... ..... ..... ..... ..... .....						
eral	-----						
Wiggum	ATGCCAGTAGTAACCCGCTTGATTCTGTTTGAAGTGTGTAGGGCTCAGACTTGACCGAGTGGACTCAATC						
PPI-Soy-FTB	-----						
DuP-Soy-FTB	-----						
PPI-Corn-FTB	-----						
DuP-Corn-FTB	-----						
Pea FT-B	-----						
Tomato	-----GTAAACGAGCGTTGATT						
Tobacco	-----						

	80	90	100	110	120	130	140
PPI-BnFTb	..... ..... ..... ..... ..... ..... ..... .....						
eral	-----						
Wiggum	GGCGAATCTGTACGGAGGACACGGGAATCAACGCGGCGAGAGTGATGGAAGAGCTTTCAAGCCTAAC						
PPI-Soy-FTB	-----						
DuP-Soy-FTB	-----						
PPI-Corn-FTB	-----GGCGGATCCCGACCTACCGAGGCTCAC						
DuP-Corn-FTB	-----GGCGGATCCCGACCTACCGAGGCTCAC						
Pea FT-B	-----CGGACCCCCCGTCCACAATCGTGAT						
Tomato	GTCGCTGACGAAATTTACAGTCAAGAGTAGTAACCGGTTGTAGTGAAAAAATGGAGTCGAGGAAAGTGAC						
Tobacco	-----GGCACGAGCGGC-AC						

	150	160	170	180	190	200	210
PPI-BnFTb	..... ..... ..... ..... ..... ..... ..... .....						
eral	-----						
Wiggum	CGTCAAGTCAGCGCGAGCAATTTCTGGTGGAGAACGATGTGTTCGGGATCTATAATTACTTCGACGCCAGC						
PPI-Soy-FTB	-----GCCACCATTC						
DuP-Soy-FTB	-----GCCACCATTC						



PPI-Corn-FTB GGTGACGCGAGGTGGAGCAGATGAAGGTGGAGGCCAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCC  
 DuP-Corn-FTB GGTGACGCGAGGTGGAGCAGATGAAGGTGGAGGCCAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCC  
 Pea FT-B GATGACGTCTCCGCGAGCATTCAACAACAGCTTACTCAAACCCGCGGAGTAACACATGGAAGCTTCA  
 Tomato GAAGACGCTGGAAGATCAATGGGTGGAGCGTCGAGTCCGAGAGATATACGATTATTTCTACAGCATT  
 Tobacco GAGGACACTGGAAGATCAATGGATGGTGGAGCGTCAAGTTCGGGAGATATACAATTTTCTACAGCATT

220 230 240 250 260 270 280  
 PPI-BnFTb .....-ATCGAGATTACCGAGATAACCAATT-GGATTATC-----TCATGA  
 eral .....-ATCGAGATTACCGAGATAACCAATT-GGATTATC-----TCATGA  
 Wiggum .....-ATCGAGATTACCGAGATAACCAATT-GGATTATC-----TCATGA  
 PPI-Soy-FTB CTCGCAACGCCCAAACCTCAT-GTTGGAGCTTCAACCGSATAATCAGAT-GCAGTAT-----GTCTCCA  
 DuP-Soy-FTB CTCGCAACGCCCAAACCTCAT-GTTGGAGCTTCAACCGSATAATCAGAT-GCAGTAT-----GTCTCCA  
 PPI-Corn-FTB GCGGCCAACACGAAATCCATCATGCTAGAGCTGTGGCGTGATCAGCATAT-CGAGTATG-----TGACGC  
 DuP-Corn-FTB GCGGCCAACACGAAATCCATCATGCTAGAGCTGTGGCGTGATCAGCATAT-CGAGTATG-----TGACGC  
 Pea FT-B ACCGCGGCGGAGACCCAACTCCGAGCTGAGTCCGAGAGATCATGGATAGTAGAATACAGGCTCTTTC  
 Tomato TCCGCCAACTCTCCGTCCGACCTCATAGAGATCGAACCCTGACAAACACTT-CGATTATG-----TAAGCC  
 Tobacco CCNCCCAATTG-----CCACTTAGAGACTTCAACAGAAACCACTT-CGATTATG-----TCATCTC

290 300 310 320 330 340 350  
 PPI-BnFTb .....-TGGCTTTGTTACTG  
 eral AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG  
 Wiggum AAGGCTTAAGGCAGCTT---GGTCCGCGAGTTTCTTCCTTAGATGCTAATCGACCTTGGCTTTGTTACTG  
 PPI-Soy-FTB AAGGCTTCCCACTCTC---AGTTCCCGATTTCCTTTGGAGCGCTAATCGACCTTGGCTTTGTTACTG  
 DuP-Soy-FTB AAGGCTTCCCACTCTC---AGTTCCCGATTTCCTTTGGAGCGCTAATCGACCTTGGCTTTGTTACTG  
 PPI-Corn-FTB CTGGCTCAGGCATATG---GGACCGAGCTTTCATGTTCTAGATGCCAATCGCCCTTGGCTATGCTACTG  
 DuP-Corn-FTB CTGGCTCAGGCATATG---GGACCGAGCTTTCATGTTCTAGATGCCAATCGCCCTTGGCTATGCTACTG  
 Pea FT-B ATATTATCAACTCTTCGCCAATATTCTCTTAACCCCAATCTATCATTCGACCTTGGCTTGTGTTACTG  
 Tomato AAGGCTCAGAAACTT---GGTCCGCTGTTTCTCCTTTGATGCCACTCGACCATGGCTTTGCTACTG  
 Tobacco GAGGCTCAGAAACTT---GGTCCGCTGTTTCTCCTTTGATGCCACTCGACCATGGCTTTGCTACTG

360 370 380 390 400 410 420  
 PPI-BnFTb GATTCTTCATTCAATTGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 eral GATTCTTCATTCAATTGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 Wiggum GATTCTTCATTCAATTGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 PPI-Soy-FTB GATCTTCCATCCATTGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 DuP-Soy-FTB GATCTTCCATCCATTGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 PPI-Corn-FTB GATGCTTCATCCACTTGGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 DuP-Corn-FTB GATGCTTCATCCACTTGGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 Pea FT-B GATTATTCAATTGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 Tomato GACACTTCATTCAATCGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT  
 Tobacco GATACTTCATTCAATCGCTTTGCTTGGGAGTCTCTCGATGATGACTTAGAAAACAATGCAATCGATT

430 440 450 460 470 480 490  
 PPI-BnFTb CTTGGACGTTGCCAGGGCTTCTGATGGTGGATATGGTGGTGGCTCCTGGCCAACTTCCACATCTTGCAAGAA  
 eral CTTGGACGTTGCCAGGGCTTCTGATGGTGGATATGGTGGTGGCTCCTGGCCAACTTCCACATCTTGCAAGAA  
 Wiggum CTTGGACGTTGCCAGGGCTTCTGATGGTGGATATGGTGGTGGCTCCTGGCCAACTTCCACATCTTGCAAGAA  
 PPI-Soy-FTB CTTAACCCTTGCCAGGATCGGAATGGTGGATATCGCGGGGACCGGCGAGATGCTCATATTGCCAGAA  
 DuP-Soy-FTB CTTAACCCTTGCCAGGATCGGAATGGTGGATATCGCGGGGACCGGCGAGATGCTCATATTGCCAGAA  
 PPI-Corn-FTB TTAGCTCGATGTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTTGCTTCACTAGCTAGCA  
 DuP-Corn-FTB TTAGCTCGATGTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGGACAGTTGCTTCACTAGCTAGCA  
 Pea FT-B CTTAACCCTTGCCAGGATCGGAATGGTGGATATCGCGGGGACCTGGTCAATGCTCATCTTGCCAGAA  
 Tomato CTGACCCGTTGCCAGGATAAAGATGGTGGCTATGGAAGTGGACCTGGTCAGATGCTCATCTTGCAAGTA  
 Tobacco CTGAGCCGTTGCCAGGATGAAATGGTGGCTATGGAAGTGGACCTGGTCAGATGCTCATCTTGCAAGTA

500 510 520 530 540 550 560  
 PPI-BnFTb GTTATGCTGCAGTCAATACACTTCTTACTTTAGGAGGTGACAAAGCCCTTCTTCAATTAATAGAGAAAA  
 eral GTTATGCTGCAGTCAATACACTTCTTACTTTAGGAGGTGACAAAGCCCTTCTTCAATTAATAGAGAAAA  
 Wiggum GTTATGCTGCAGTCAATACACTTCTTACTTTAGGAGGTGACAAAGCCCTTCTTCAATTAATAGAGAAAA  
 PPI-Soy-FTB CTTATGCTGCTGTTAATTCACTTATTACTTTTGGTGGTGAAGAAATCCCTGGCATCAATTAATAGAGATAA  
 DuP-Soy-FTB CTTATGCTGCTGTTAATTCACTTATTACTTTTGGTGGTGAAGAAATCCCTGGCATCAATTAATAGAGATAA  
 PPI-Corn-FTB CTTATGCTGCTGTTAATTCACTTCTCAAAATAGGAGCCGAAAGAGCATTGTCTCATCAATCAATAGGGGCAA  
 DuP-Corn-FTB CTTATGCTGCTGTTAATTCACTTCTCAAAATAGGAGCCGAAAGAGCATTGTCTCATCAATCAATAGGGGCAA  
 Pea FT-B CTTATGCTGCAGTCAATACACTTCTTACTTTTGGTGGTGAAGAAATCTTGGCATCTTAAATAGAGAAAA  
 Tomato CTTATGCTGCAGTCAATACACTTCTTACTTTTGGGCAACCTGAAGCTCTGTCTCATCAATTAATAGAGAAAA  
 Tobacco CTTATGCTGCAGTCAATACACTTCTTACTTTTGGGCAACCTGAAGCTCTGTCTCATCAATTAATAGAGAAAA

570 580 590 600 610 620 630  
 PPI-BnFTb AATGGCTTCTTTCTTAAGACGAATGAAGGATACAAATGGAGGTTTCAGGATGCATATATGGGAGAAATA  
 eral AATGTCTTCTTTCTTAAGACGAATGAAGGATACAAATGGAGGTTTCAGGATGCATATATGGGAGAAATA  
 Wiggum AATGTCTTCTTTCTTAAGACGAATGAAGGATACAAATGGAGGTTTCAGGATGCATATATGGGAGAAATG  
 PPI-Soy-FTB ACTGTATCTGTTCTGCGCGGATGAAGCAACCAAAATGGTGGATTTCAGGATGCATATATGGGAGAAATG

DuP-Soy-FTB ACTGTATCGCTTTCTGCGCGGATGAAGCAACCAATGGTGGATTTCAGGATGCATGATGAAGGTGAAATT  
 PPI-Corn-FTB CCTGTACAAATTTTATGCTGCGATGAAGAGATGATCAGGTGCTTTCAGAAATGCATGATGGTGGCGAAATT  
 DuP-Corn-FTB CCTGTACAAATTTTATGCTGCGATGAAGAGATGATCAGGTGCTTTCAGAAATGCATGATGGTGGCGAAATT  
 Pea FT-B GTTGTACCGCTTTATGCGCGGATGAAGCAACCAACCGCGGATTTCAGGATGCATGACGAGGAGAAATT  
 Tomato GTTGTACACATTTTATGCTGCGAATGAAGAGCGCAAGTGGTGGATTTCAGGATGCATGATGGTGGAGAGTA  
 Tobacco ATTGTATACATTTTGGGTGCAATGAAGAGCAAACTGGTGGCTTCAGGATGCATGATGGTGGAGAGTA

640 650 660 670 680 690 700  
 PPI-BnFTb GATGTCCGAGCGTGCTACACTGCGATTGTTGATTGCAAGCATCCTGAACATTGTTGGATGATGAACCTACCC  
 eral GATGTTTCGTGCATGCTACACTGCAATTTCTGTTGCAAGCATCCTAAATATTATGGATGATGAACCTACCC  
 Wiggum GATGTTTCGTGCATGCTACACTGCAATTTCTGTTGCAAGCATCCTAAATATTATGGATGATGAACCTACCC  
 PPI-Soy-FTB GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC  
 DuP-Soy-FTB GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC  
 PPI-Corn-FTB GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC  
 DuP-Corn-FTB GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC  
 Pea FT-B GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC  
 Tomato GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC  
 Tobacco GATGTTTCGAGCTTGCTACACTGCAATTTCTGTTGCAAGTGTGTTGAACATTGTTGGATGATGAGCTGATCC

710 720 730 740 750 760 770  
 PPI-BnFTb GCGGCTTAGGAGATTACATTTTCAGTTGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGA  
 eral AGGGCCTTAGGAGATTACATTTTCAGTTGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGA  
 Wiggum AGGGCCTTAGGAGATTACATTTTCAGTTGCCAAACTTATGAAGGTGGCATTGGAGGGGAACCTGGCTCCGA  
 PPI-Soy-FTB AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAAGGTGGCATTGGCTGGTGAGCCTGGTTCTGA  
 DuP-Soy-FTB AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAAGGTGGCATTGGCTGGTGAGCCTGGTTCTGA  
 PPI-Corn-FTB AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAAGGTGGCATTGGCTGGTGAGCCTGGTTCTGA  
 DuP-Corn-FTB AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAAGGTGGCATTGGCTGGTGAGCCTGGTTCTGA  
 Pea FT-B AGAATGTTGGAGACTACATTATAAGCTGTCAAACATATGAAGGTGGCATTGGCTGGTGAGCCTGGTTCTGA  
 Tomato ATGGTGTGGAAATACATCCTAAGTTGTCAAGCTTATGAAGGTGGAAATGGCTGGCGAACCAGGTTCTGA  
 Tobacco ATGATGTTGGAAATACATCCTAAGTTGTCAAGCTTATGAAGGTGGAAATGGCTGGCGAACCAGGTTCTGA

780 790 800 810 820 830 840  
 PPI-BnFTb AGCTCATGGTGGGTACACCTTACTGTGGCTTGGCTACTATGATTTAATCAATGAAAGTCAACCGCTTGAAT  
 eral AGCTCATGGTGGGTATACCTACTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 Wiggum AGCTCATGGTGGGTATACCTACTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 PPI-Soy-FTB GGCTCATGGTGGGTACACCTTACTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 DuP-Soy-FTB GGCTCATGGTGGGTACACCTTACTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 PPI-Corn-FTB AGCAGATGGTGGGTATACCTTCTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 DuP-Corn-FTB AGCAGATGGTGGGTATACCTTCTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 Pea FT-B AGCAGATGGTGGGTATACCTTCTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 Tomato AGCTCATGGTGGGTATACCTTCTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT  
 Tobacco AGCTCATGGTGGGTATACCTTCTGTGGCTTGGCTACTATGATTTAATCAATGAGGTCAACCGCTTGAAT

850 860 870 880 890 900 910  
 PPI-BnFTb TTGGATTCTTAATGAATTGGGTGTTGATCGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 eral TTGGATTCTTAATGAATTGGGTGTTGATCGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 Wiggum TTGGATTCTTAATGAATTGGGTGTTGATCGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 PPI-Soy-FTB CTGCCTCGATTGATTGATCGGGTGGCTTTCCGACAAAGGTAAGGAATGTTGGATTCCAAGGTAGCAGCAACA  
 DuP-Soy-FTB CTGCCTCGATTGATTGATCGGGTGGCTTTCCGACAAAGGTAAGGAATGTTGGATTCCAAGGTAGCAGCAACA  
 PPI-Corn-FTB TTGCCTAGTTGATTGATCGGGTGGCTTTCCGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 DuP-Corn-FTB TTGCCTAGTTGATTGATCGGGTGGCTTTCCGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 Pea FT-B CTGCCTCGATTGATTGATCGGGTGGCTTTCCGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 Tomato TTGCCAGGTTTAATTGATTGGGTGGTATTTAGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA  
 Tobacco TTGCCAGGTTTAATTGATTGGGTGGTATTTAGACAAAGGAGTAGAAATGGATTCCAAGGTAGCAGCAACA

920 930 940 950 960 970 980  
 PPI-BnFTb AATTGGTTCGACCGTTGCTACACCTTTTGGCAGGCGAGCCCTGTTGTTCTACTACAGCGATTTTTTCATC  
 eral AATTGGTTCGATGGTTGCTACACCTTTTGGCAGGCGAGCCCTGTTGTTCTACTACAAAGATTATATTCAAC  
 Wiggum AATTGGTTCGATGGTTGCTACACCTTTTGGCAGGCGAGCCCTGTTGTTCTACTACAAAGATTATATTCAAC  
 PPI-Soy-FTB AACTGGTTCGATGGTTGCTATTCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAAGATTATCTTCTAT  
 DuP-Soy-FTB AACTGGTTCGATGGTTGCTATTCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAAGATTATCTTCTAT  
 PPI-Corn-FTB AATTGGTTCGATGGTTGCTACTCCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAACAAAGTTAATTACGAT  
 DuP-Corn-FTB AATTGGTTCGATGGTTGCTACTCCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAACAAAGTTAATTACGAT  
 Pea FT-B AATTGGTTCGATGGTTGCTACTCCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAAGATTATCTTCTAT  
 Tomato AATTGGTTCGATGGTTGCTACTCCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAACAAAGTTAATTACGAT  
 Tobacco AATTAGTTCGATGGTTGCTATTCTTTTGGCAGGCGAGCTGCTGTTGCTCTATTCAACAAAGTTAATTACGAT

990 1000 1010 1020 1030 1040 1050  
 PPI-BnFTb CCAGGAT-ATGGCACC-----TCATGGATCATCATCA-----CATAATGTCACAAGGGACAGAT  
 eral CAATGATCATGACG-----TCATGGATCATCATCA-----CATAATGTCACAAGGGACAAAT  
 Wiggum CAATGATCATGACG-----TCATGGATCATCATCA-----CATAATGTCACAAGGGACAAAT

PPI-Soy-FTB	TATCAACAAACAGATG-----GAACAGA-CATCA-----CAGATTTTTCGGTATCTTAT
DuP-Soy-FTB	TATCAACAAACAGATG-----GAAAGAGA-CATCA-----CAGATTTTTCGGTATCTTAT
PPI-Corn-FTB	TGTTGATAAGCAATTGAGGTCCTCGTA-----T-----TCTGCAAAA-----GG
DuP-Corn-FTB	TGTTGATAAGCAATTGAGGTCCTCGTA-----T-----TCTGCAAAA-----GG
Pea FT-B	TATCCAGCAACAAATG-----GCAGAGG-CATCA-----CAGTTTGTACAGTATCTGAT
Tomato	AGTCCATCAACAACTAGGGCTGTCAAAATGAGCTCAGTACAGAAAGTGCTGATGATTCTTCAGAGTCAGAG
Tobacco	AGTCCATCAACAACTAGGGCTGTCAAAATGAGCTCAGTACAGAAAGTGCTGATGATTCTTCAGAGTCAGAG
	1060 1070 1080 1090 1100 1110 1120
PPI-BnFTb	GAAGATCAACAGGA-ACATGGTCATCATGAAGATGATCCTGAAGACAGTGATGAAGATGA---TTCTGAT
eral	GAAGAACAT-----CATGCTCATCATGAAGATGACCTTGAAGACAGTGATGATGATGATGATTCTGAT
Wiggum	GAAGAACAT-----CATGCTCATCATGAAGATGACCTTGAAGACAGTGATGATGATGATGATTCTGAT
PPI-Soy-FTB	GTATCTGAAG-----CAAAAGAAATTTGGATGGAACCTCTACTCA-TGCAACATGCCGTGGTGAGCAT
DuP-Soy-FTB	GTATCTGAAG-----CAAAAGAAATTTGGATGGAACCTCTACTCA-TGCAACATGCCGTGGTGAGCAT
PPI-Corn-FTB	CCATCAGGAGAG-----GATGCCCTGCAG-----CACCAATTCTATAT---GGGTGCACC-----G-CGA
DuP-Corn-FTB	CCATCAGGAGAG-----GATGCCCTGCAG-----CACCAATTCTATAT---GGGTGCACC-----G-CGA
Pea FT-B	GCACCTGAAG-----AAAAGGAATTTTGGACGSAACCTCAAGTCA-TCCAACCTCCCATATTAGGCAT
Tomato	TTATCTGATGAAGAAGAGCATTGGAGGGATATCCTCTCATGTTCA-CGATACCTTCCCTCTGGACAA
Tobacco	TTATCTGATGAAG-----GAGCATTGCAAGGGACATCATCTCATGTTCA-GAAGACTTGCCCTCTGGACAA
	1130 1140 1150 1160 1170 1180 1190
PPI-BnFTb	GAGGAT-----AGCGATGAA---GATTGAGGAATGGTCAACCAAGTTTCATCATACCTCTAC-CTAC
eral	GAGGAC-----AACGATGAA---GATTGAGGAATGGTCAACCAAGTTTCATCATACCTCTAC-CTAC
Wiggum	GAGGAC-----AACGATGAA---GATTGAGGAATGGTCAACCAAGTTTCATCATACCTCTAC-CTAC
PPI-Soy-FTB	GAAGGC-----ACCAGTGAATCGAGTTCTGATTTTAAAAATATTGCCATATAAATTTAT-TAAT
DuP-Soy-FTB	GAAGGC-----ACCAGTGAATCGAGTTCTGATTTTAAAAATATTGCCATATAAATTTAT-TAAT
PPI-Corn-FTB	ATAACT-----CTTCTCTCTCTGTTGACTATGCCAAGTTTGATTTTATTTATACAAC
DuP-Corn-FTB	ATAACT-----CTTCTCTCTCTGTTGACTATGCCAAGTTTGATTTTATTTATACAAC
Pea FT-B	GAAGGC-----ATGAATGAATCTGCTCATCTGACGTTAAAAATATTGTTTATAAATTTAT-TAGT
Tomato	GCAGTGCTTGTCAAGAAATGCTTCTCATAGCCCAAAATAGCAGATATCTGATATGATTTTAT-CAAC
Tobacco	GAAGGA-----CAGGAAATGCTTCTGATCTCCACAAAGATAGCAGATATCTGTTATGATTTTGT-CAAT
	1200 1210 1220 1230 1240 1250 1260
PPI-BnFTb	ATTGACAGGAGAAATCAACCTGTTTTGATAGCCTCGGCTTGCAAGATATGCTCTTTGCTCTCAGG
eral	ATTAACAGGAGAAATGCAAGTGGTTTTGATAGCCTCGGCTTGCAAGATATGCTCTTTGCTCTCAGG
Wiggum	ATTAACAGGAGAAATGCAAGTGGTTTTGATAGCCTCGGCTTGCAAGATATGCTCTTTGCTCTCAGG
PPI-Soy-FTB	GAGTGGAGAGCACAAGAACCACTTTTTCAGAGTATGCTTTACAGCAATATATCTCTTATCTGCACAGG
DuP-Soy-FTB	GAGTGGAGAGCACAAGAACCACTTTTTCAGAGTATGCTTTACAGCAATATATCTCTTATCTGCACAGG
PPI-Corn-FTB	AGAGCAACCAA-ATTGGGCCACTCTTCCATACATTGCCCCTGCAACAATACATCCTACTTTGCTCTCAGG
DuP-Corn-FTB	AGAGCAACCAA-ATTGGGCCACTCTTCCATACATTGCCCCTGCAACAATACATCCTACTTTGCTCTCAGG
Pea FT-B	GAGTGGAGACAAATGAACCACTTTTTCAGAGCATTGCTTTACAGCAATATATCTCTTATCTGCACAGG
Tomato	CGACCCATAGCTATGAGGCTCTCTTTGAGAGCATGTATCTGCAGCAATATGTTCTTCTTGTCTCTCAGG
Tobacco	CGNACGATAGCTATGCGACCTGTTTTGACAGCTTTATCTGCAGCAATACGTTCTCTCTGCTCCGAGG
	1270 1280 1290 1300 1310 1320 1330
PPI-BnFTb	TTGCTGATGGTGGATTTCAGAGACAACTGAGCAAAACCCCTGACTTCTACCAACATGTTACTGCCATAAG
eral	TCCCTGACGGTGGATTTCAGAGACAACTGAGCAAAACCCCTGACTTCTACCAACATGTTACTGCCATAAG
Wiggum	TCCCTGACGGTGGATTTCAGAGACAACTGAGCAAAACCCCTGACTTCTACCAACATGTTACTGCCATAAG
PPI-Soy-FTB	AGCAAGAGGGTGGACTGAGAGACAAACCGGTAACGTAAGAGATCATTATCACACATGTTACTGTTTAAG
DuP-Soy-FTB	AGCAAGAGGGTGGACTGAGAGACAAACCGGTAACGTAAGAGATCATTATCACACATGTTACTGTTTAAG
PPI-Corn-FTB	TACTAGAGGGAGGCTTGAGGGATAAGCCTGGAAAGAAACAGAGATCACTATCATCTATGCTGCTCAGG
DuP-Corn-FTB	TACTAGAGGGAGGCTTGAGGGATAAGCCTGGAAAGAAACAGAGATCACTATCATCTATGCTGCTCAGG
Pea FT-B	AGCAAGATGGTGGCTCAGGGACAAACCGGTAACGTAAGAGATCATTATCATCTATGCTGCTCAGG
Tomato	TTGAAGTTGGTGGTTTCAGAGACAACTGGGAAAGGGTAGAGATCACTACCAATACCTGTTACTGTTTAAG
Tobacco	T---AGATGGAGGTTTCAGAGACAACTGGGAAAGGGTAGAGATCACTACCAATACCTGTTACTGTTTAAG
	1340 1350 1360 1370 1380 1390 1400
PPI-BnFTb	CGGCTTTTCTGCTGCTCAACACGCTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGTGAATTTTG
eral	CGGCTTTTCTGCTGCTCAGCACGCTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGGACATTTTG
Wiggum	CGGCTTTTCTGCTGCTCAGCACGCTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGGACATTTTG
PPI-Soy-FTB	TGGACTCTCATTGTGCCAGTATAGTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGGACATTTTG
DuP-Soy-FTB	TGGACTCTCATTGTGCCAGTATAGTTGGTCAAAAGACGAGGACACTCCTCCTTTGACTCGGACATTTTG
PPI-Corn-FTB	TGGCTCTCGAGTTAGCCAGTACAGTGCCATGACTGATCTGTTGCTGCCATTACCTCAGCATGTCCTT
DuP-Corn-FTB	TGGCTCTCGAGTTAGCCAGTACAGTGCCATGACTGATCTGTTGCTGCCATTACCTCAGCATGTCCTT
Pea FT-B	TGGTCTTCAATTGCTCAGTATAGCTGGAGCCAGCAAGCTGATTCTAGACCATTACCCAGGGATGATTTT
Tomato	TGGTCTTCAATTGCTCAGTATAGCTGGAGCCAGCAAGCTGATTCTAGACCATTACCCAGGGATGATTTT
Tobacco	TGGTCTTCAATTGCTCAGTATAGCTGGAGCCAGCAAGCTGATTCTAGACCATTACCCAGGGATGATTTT
	1410 1420 1430 1440 1450 1460 1470
PPI-BnFTb	GGTGGCTACCCA-AA--CCACCTTGAACCTGTTCACTCTCCAGAACATTGTTCTGGATCGGTATTTATG
eral	GGTGGCTACTCG-AA--TCTCCTTGAACCTGTTCACTCTCCAGAACATTGTTCTGGATCGGTATTTATG

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PPI-BnFTb	..... .....
eral	-----
Wiggum	-----
PPI-Soy-FTB	-----
DuP-Soy-FTB	-----
PPI-Corn-FTB	-----
DuP-Corn-FTB	-----
Pea FT-B	-----
Tomato	AAAAAAAAA
Tobacco	-----

- 1) PPI-BnFTB; FT3 (SEQ ID NO:15)
- 2) era1 (SEQ ID NO:2)
- 3) Wiggum (SEQ ID NO:87)
- 4) PPI-Soy-FTB; FT5 (SEQ ID NO:42)
- 5) DuP-Soy-FTB (SEQ ID NO:88)
- 6) PPI-Corn-FTB; FT6 (SEQ ID NO:45)
- 7) DuP-Corn-FTB (SEQ ID NO:89)
- 8) Pea-FT-B (SEQ ID NO:90)
- 9) Tomato (SEQ ID NO:91)
- 10) Tobacco (SEQ ID NO:92)

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DuP-Corn-FTB	DFLARCQDKGGYSGGPGQMPHLATTYAAVNLTITGSSRALSSINRGNLYNEMLOMKDVSQAFRMHDCG
Pea FT-B	DFLNRCQDPNGGYAGGPGQMPHLATTYAAVNLTITLGGKSLASINRNKLYGEMRRMKOPNGGFRMHDEG
Tomato	DFLTRCQDKGGYCGGPGQMPHLATTYAAVNSLTITLGPALSSINRKLITFLRMKDASGGFRMHDCG
Tobacco	DFLSRCQDEGGYCGGPGQMPHLATTYAAVNSLTITLGPALSSINRKLITFLWLOMKDITSGGFRMHDCG
	220 230 240 250 260 270 280
PPI-BnFTB	EIDVRACYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEVDR
eral	EIDVRACYTAISVASTLNIMODELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEVER
Wiggum	EMDVRACYTAISVASTLNIMODELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEVDR
PPI-Soy-FTB	EIDVRACYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILIGEVNH
DuP-Soy-FTB	EIDVRACYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILIGEVNH
PPI-Corn-FTB	EIDVRASYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEASK
DuP-Corn-FTB	EIDVRASYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEASK
Pea FT-B	EIDVRACYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILIGEVNH
Tomato	EIDVRACYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEVDR
Tobacco	EIDVRACYTAISVASTLNIVDDELTGCGDYILSCQTYEGGICGEPGSEAHGGYTCGLAAMILINEVDR
	290 300 310 320 330 340 350
PPI-BnFTB	LNLDSLNNWVVRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRFPSQDMAPHGSSSHMEOGTDEDEH
eral	LNLDSLNNWVVRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
Wiggum	LNLDSLNNWVVRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
PPI-Soy-FTB	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
DuP-Soy-FTB	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
PPI-Corn-FTB	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
DuP-Corn-FTB	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
Pea FT-B	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
Tomato	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
Tobacco	LDLPLRLVDVWVFRQGVEMGFGQRTNKLVDGCTFWQAAAPCVLLQRLYSTNOHDVHG-SSHISEGNTNEEH-
	360 370 380 390 400 410 420
PPI-BnFTB	EHGHDED-DPE--DSDEDD-S--DEDS--DEDSNGHGHHT-STYIDR--RTQPVFDSGLQRYVLLCS
eral	-HAHDED-DLE--DSDDDDDS--DEDN--DEDSVNGHRTHT-STYINR--RMQLVFDPSGLQRYVLLCS
Wiggum	-HAHDED-DLE--DSDDDDDS--DEDN--DEDSVNGHRTHT-STYINR--RMQLVFDPSGLQRYVLLCS
PPI-Soy-FTB	-----KE-SLDGTSSSHATCRG--EHG--TSESSSDFKNIAMKFINEWRAQEPLEHSTALQOQYILLCS
DuP-Soy-FTB	-----KE-SLDGTSSSHATCRG--EHG--TSESSSDFKNIAMKFINEWRAQEPLEHSTALQOQYILLCS
PPI-Corn-FTB	-----TSSYGCTAN-----KSSSAVDYAKFGEDFIQSSNOICPLPHNTALQOQYILLCS
DuP-Corn-FTB	-----TSSYGCTAK-----KSSSAVDYAKFGEDFIQSSNOICPLPHNTALQOQYILLCS
Pea FT-B	-----KE-CLDGTSSSHATSHI--RHEG--MNESCSDDVKNIGYFISEWRQSEPLEHSTALQOQYILLCS
Tomato	SELSDEREHLEGITSSHVQDTFPLGQAGACQENASHSPKADTGMDFINRPIAMRPLFDSMYLQOQYILLCS
Tobacco	SELSDEE-HIQGTSSHVQKTCPGQEG--QENASDPTKTADTGMDFINRPIAMRPLFDSMYLQOQYILLCS
	430 440 450 460 470 480 490
PPI-BnFTB	QVADGGFRDKLRKPRDFYHTCYCLSGLSVAQHAWLKDEDTPPLTRDITCGYAN-HLEFVHLHNHNVMDRY
eral	KIPDGGFRDKPRKPRDFYHTCYCLSGLSVAQHAWLKDEDTPPLTRDITCGYAN-HLEFVQLHNVMDRY
Wiggum	KIPDGGFRDKPRKPRDFYHTCYCLSGLSVAQHAWLKDEDTPPLTRDITCGYAN-HLEFVQLHNVMDRY
PPI-Soy-FTB	QEQEGGLRDKPGKRRDHYHTCYCLSGLSLQYSSWSKHPDSPP-----
DuP-Soy-FTB	QEQEGGLRDKPGKRRDHYHTCYCLSGLSLQYSSWSKHPDSPP-----
PPI-Corn-FTB	QVLEGGLRDKPGKRRDHYHSCYCLSGLSVAQYSAMTETGSCPLPQHVLGFPYSN-LLEFPIH-----
DuP-Corn-FTB	QVLEGGLRDKPGKRRDHYHSCYCLSGLSVAQYSAMTETGSCPLPQHVLGFPYSN-LLEFPIH-----
Pea FT-B	QEQEGGLRDKPGKRRDHYHSCYCLSGLSLQYSSWSKRPPSPPLPKVVMGFPYSNLLLEPIHPFNVMDRY
Tomato	QHEVGGFRDKPGKRRDHYHTCYCLSGLSLQYSSWDEADSTPLPRDVFQFPYSKCLLEOVHPLFNVMDRY
Tobacco	QID-GGFRDKPGKRRDHYHTCYCLSGLSLQYSSWNEADAPPLPRDVFQFPYSNLLLEPIHPFNVMDRY
	500 510
PPI-BnFTB	YEASRF-----
eral	NEAIEEFFKAA-----
Wiggum	NEAIEEFFKAA-----
PPI-Soy-FTB	-----
DuP-Soy-FTB	-----
PPI-Corn-FTB	-----
DuP-Corn-FTB	-----
Pea FT-B	REAHFFFSQL-----
Tomato	YEAREYSQACETVSPSLAPTFSET
Tobacco	YEAREYFSCL-----

Also included in the invention is the farnesyl transferase alpha consensus sequence of SEQ ID NO:93 and the farnesyl transferase beta consensus sequence of SEQ ID NO:94 To generate the consensus sequence, the farnesyl transferase alpha and farnesyl transferase beta



sequences of the invention were aligned using the program BioEdit. The homology between the farnesyl transferase alpha (FTA) polypeptide sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10E. The homology between the farnesyl transferase beta (FTB) polypeptide sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10F.

**Table 10E ClustalW Amino Acid Analysis of FT Alpha**

	10	20	30	40	50	60	70
BnA-12	-----	-----	-----	-----	-----	MDYFRAIYFS	DERSPRALRL
At-FT-A	-----	MNFD	ETVPL	SCRE	WSDV	VEIT	QEDGPNPVVPIAYKEEFRE
PPI-Soy-FTA	MESGS	SEGE	EVQ	RVPL	R	ER	VEWSDVTPVPONDGPNPVVPIQYTEEFSEVMDYFRAVYLT
Consensus	-----	-----	VPL	R	EWS	DV	P
	80	90	100	110	120	130	140
BnA-12	TEEA	LS	LNSG	NYTV	WHF	FR	LVLELNS
At-FT-A	TEET	LL	LNSG	NYTV	WHF	FR	LVLELNS
PPI-Soy-FTA	TAE	AV	QF	NSG	NYTV	WHF	FR
Consensus	TEEA	LS	LNSG	NYTV	WHF	FR	LVLELNS
	150	160	170	180	190	200	210
BnA-12	LEKE	FTRR	VLS	SLDA	KHYH	AWSH	RQWALQALGGWE
At-FT-A	RELE	FTRR	VLS	SLDA	KHYH	AWSH	RQWALQALGGWE
PPI-Soy-FTA	NELE	FTRR	VLS	SLDA	KHYH	AWSH	RQWALQALGGWE
Consensus	LEKE	FTRR	VLS	SLDA	KHYH	AWSH	RQWALQALGGWE
	220	230	240	250	260	270	280
BnA-12	EAMRE	SEVS	YTI	KAIL	ANP	NESS	SWRYLKALYKDDTESWISDPSVSSVCLKVL
At-FT-A	EAMRE	SEVS	YTI	KAIL	ANP	NESS	SWRYLKALYKDDTESWISDPSVSSVCLKVL
PPI-Soy-FTA	EAMRE	SEVS	YTI	KAIL	ANP	NESS	SWRYLKALYKDDTESWISDPSVSSVCLKVL
Consensus	EAMRE	SEVS	YTI	KAIL	ANP	NESS	SWRYLKALYKDDTESWISDPSVSSVCLKVL
	290	300	310	320	330	340	350
BnA-12	LCDGL	RPTN	EH	RDSV	KALAN	-----	EEPETNL
At-FT-A	LCDGL	RPTN	EH	RDSV	KALAN	-----	EEPETNL
PPI-Soy-FTA	LCFC	YCPN	EE	IRDA	YDAIK	TADMDKQDLDDDEKGE	SONLN
Consensus	LCDGL	RPTN	EH	RDSV	KALAN	-----	EEPETNL
BnA-12	--	(SEQ ID NO:13)					
At-FT-A	AI	(SEQ ID NO:8)					
PPI-Soy-FTA	--	(SEQ ID NO:39)					
Consensus	--	(SEQ ID NO:93)					

**Table 10F ClustalW Amino Acid Analysis of FT Beta**

	10	20	30	40	50	60	70
PPI-BnFTB	-----	-----	-----	-----	-----	-----	-----
PPI-Soy-FTB	-----	ATTP	-----	-----	-----	-----	-----
PPI-Corn-FTB	ADPDL	PR	LT	VT	VEQ	MK	VEARV
Consensus	-----	ATTP	-----	-----	-----	-----	-----
	80	90	100	110	120	130	140
PPI-BnFTB	-WLCY	WIF	HS	IALL	GESV	DDLE	NNAID
PPI-Soy-FTB	PWLCY	WIF	HS	IALL	GESV	DDLE	NNAID
PPI-Corn-FTB	PWLCY	WIF	HS	IALL	GESV	DDLE	NNAID
Consensus	-WLCY	WIF	HS	IALL	GESV	DDLE	NNAID
	150	160	170	180	190	200	210
PPI-BnFTB	-----	-----	-----	-----	-----	-----	-----
PPI-Soy-FTB	-----	-----	-----	-----	-----	-----	-----
PPI-Corn-FTB	-----	-----	-----	-----	-----	-----	-----
Consensus	-----	-----	-----	-----	-----	-----	-----

Also included in the invention is the farnesyl transferase alpha consensus sequence of SEQ ID NO:95 and the farnesyl transferase beta consensus sequence of SEQ ID NO:96. To generate the consensus sequence, the farnesyl transferase alpha and farnesyl transferase beta sequences of the invention were aligned using the program BioEdit. The homology between the farnesyl transferase alpha (FTA) nucleic acid sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10G. The homology between the farnesyl transferase beta (FTB) nucleic acid sequences of the invention is shown graphically in the ClustalW analysis shown in Table 10H.

[illegible]



BnA-12	CGACGAGCGTCTCTCGCGCGCTGCGACTCACGGAAGAAGCTCTCCGCTTAAACTCCGG	89
At-FT-A	CGACGAGCGATCTCTCGCGCGCTACGACTCACGGAAGAAACCTCTCTTAAACTCCGG	227
PPI-Soy-FTA	CGATGAACCGCTCCCTCGCGCGCTCGCTCTCACAGCCGAAGCCSTTCAATTCAACTCCGG	239
Consensus	CGACGAGCGTCTCTCTCGCGCGCTGCGACTCACGGAAGAAGCCCTCCCTTAAACTCCGG	167
250 260 270 280 290 300		
BnA-12	CAACTACACCGTGTGGCATTTCGGCGCTTAGTACTCGAGGAGCTTAATAACGACTTGTA	149
At-FT-A	CAACTACACAGTGTGGCATTTCAGCGCGCTTAGTACTCGAGGCGCTTAATCAGACTTGTT	287
PPI-Soy-FTA	CAACTACACGTGTGGCATTTCGACCGTTGTACTTGACTTCGCTTAAAGTCGACTTGAA	299
Consensus	CAACTACACGTGTGGCATTTCGGCGCTTAGTACTCGAGGCGCTTAATACGACTTGTA	224
310 320 330 340 350 360		
BnA-12	TGAAGAGCTCAAGTTCATCGAAGCGATTGCTGAGGATAACTCTAAGAACTACCAGTTGTC	209
At-FT-A	TGAAGAACTCGAGTTCATCGAAGCGATTGCTGAGGATAACTCTAAGAACTACCAACTGTG	347
PPI-Soy-FTA	CGATTGAAGTTCGAGTTGTTCGACCGTATGGCGCTCGAAGTTCTAAAGTTATCAGATGTG	359
Consensus	TGAAGAACTCGAGTTCATCGAAGCGATTGCTGAGGATAACTCTAAGAACTACCAGTGTG	283
370 380 390 400 410 420		
BnA-12	C-----CATCATCGACGATGGGTGCGAGAGAACTGGGTCTGATGTTGCAGG	257
At-FT-A	G-----CATCATCGGCGATGGGTGCGAGAGAACTGGGTCTGATGTTGCAGG	395
PPI-Soy-FTA	NATGTTCTGTAGGCATCCCTAGACGATGGGTGCGAGAGTTAGGTCTGAAGCTAGAAA	419
Consensus	C-----CATCATCGACGATGGGTGCGAGAGAACTGGGTCTGATGTTGCAGG	331
430 440 450 460 470 480		
BnA-12	AAAGGAACTTGAGTTTACTCGGAGGGTACTATCACTTGATGCCAAACATTATCATGCTTG	317
At-FT-A	GAGAGAACTTGAAATTTACCCGTAGAGTACTTTCATTGATGCCAAACATTATCATGCTTG	455
PPI-Soy-FTA	CAATGAGCTCGAGTTCAACAAAAGATACTGTCGGTTGATGCCAAACATTATCATGCTTG	479
Consensus	AAAGAACTTGAGTTTACCCGAGGGTACTTCACTTGATGCCAAACATTATCATGCTTG	387
490 500 510 520 530 540		
BnA-12	GTCACATAGGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAGATGAGCTTAATCTACTG	377
At-FT-A	GTCACATAGGCAGTGGGCACTACGGGCATTAGGAGGATGGGAAGATGAGCTCGATTACTG	515
PPI-Soy-FTA	GTCATCATAGACAGTGGGCTCTTCAAACACTAGGAGGATGGGAAGATGAACCTTAATTATG	539
Consensus	GTCACATAGGCAGTGGGCGCTACAAGCATTAGGAGGATGGGAAGATGAGCTTAATTACTG	446
550 560 570 580 590 600		
BnA-12	CCACGAGCTCCTTGAAGCTGACGTCTTTAACAACCTCTGCAATGGAATCAGAGGTATTACGT	437
At-FT-A	TCACGAGCTCCTTGAAGCTGACGTCTTTAACAATTCGGCTGGAATCAGAGGTATTATCT	575
PPI-Soy-FTA	CACAGAACTACTTAAGAAAGACATTTTAAACAATCTGCAATGGAATCAGAGATATTGT	599
Consensus	CCACGAGCTCCTTGAAGCTGACGTCTTTAACAATCTGCAATGGAATCAGAGGTATTATGT	505
610 620 630 640 650 660		
BnA-12	TATAAGTAGATCACTTCTTTGGGAGGCGCTAGAAGCCATGAGAGAATCTGAAGTAAGCTA	497
At-FT-A	CATCACCCAATCTCTCTTTGTTGGGAGGCGCTAGAAGCCATGAGAGAATCTGAAGTAAGCTA	635
PPI-Soy-FTA	CATAACAAGTCTCTCTTTCTGGGCGGCTTAAAGCTATGAGAGATCTGAAGTCTTTA	659
Consensus	CATAAGTAGATCTCTCTTTGTTGGGAGGCGCTAGAAGCCATGAGAGAATCTGAAGTAAGCTA	564
670 680 690 700 710 720		
BnA-12	CACAGTCAAAGCCATTTTAGCAATCCCGGAACGAGAGCTCTTGGAGGTACCTCAAAGC	557
At-FT-A	CACAATCAAAGCCATTTTAAACCAATCCTGCAACGAGAGCTGATGGCGATACCTAAAGC	695
PPI-Soy-FTA	CACCATCAAAGCCATTTATAGCCTACCTGAAAATGAAAGCTCGTGGAGATACTACGAGC	719
Consensus	CACAATCAAAGCCATTTTAGCAATCCTGAAACGAGAGCTCTGGAGATACCTAAAGC	622
730 740 750 760 770 780		
BnA-12	CCTTTACAAAGACGACACAGAGTCTTGGATTAGTGATCCAAGTGTTTCCTCAGTCTGTTT	617
At-FT-A	GCTTTACAAAGACGACAGAGATCTTGGATTAGTGATCCAAGTGTTTCCTCAGTCTGTTT	755
PPI-Soy-FTA	ACTTTTAAAGGTGAAGTACTTCATGGGTAAATGATCCTCAAGTTCTTCAGTATGCTT	779
Consensus	CCTTTACAAAGACGACACAGATCTTGGATTAGTGATCCAAGTGTTTCCTCAGTCTGTTT	679
790 800 810 820 830 840		
BnA-12	GAAAGTTCTCTACCGCGGAGCTGCTTCCATGGATTGCTCTGAGCACCTTTTGGATCT	677
At-FT-A	GAAATGTTCTATCCCGCACAGATGCTTCCATGGATTGCTCTGAGCACCTTTTGGATCT	815
PPI-Soy-FTA	AAAGATTTTGA--GAACTAAGAGCAACTAGCTGTTTGCCTTAGCATTATTTAGATCT	836
Consensus	GAAATTTCTCTACCGCAAGCTGCTTCCATGGATTGCTCTGAGCACCTTTTGGATCT	734
850 860 870 880 890 900		

BnA-12	TCTGTGCGATGGGTTGAGACCAACCAACGAGCATAGAGACTCGGTGAAAGCTCTAGCTAA	737
At-FT-A	TCTATGTGATGGACTGAGACCAACCAACGAGCATAGAGACTCAGTGAGAGCTCTAGCTAA	875
PPI-Soy-FTA	TATATGCTTTGGTTATCAACCAATGAGACATTAGAGATGCCATTGACGCCCTTAAAGAC	896
Consensus	TCTATGCGATGGTTGAGACCAACCAACGAGCATAGAGACTCGGTGAAAGCTCTAGCTAA	792
	910 920 930 940 950 960	
BnA-12	TGAAGAACCAGAGACTAACTTGGCCAATTTGGTGTGTACATTCTGTGTCTGTGTGATCC	797
At-FT-A	TGAAGAACCAGAGACTAACTTGGCCAATTTGGTGTGTACTATTCTTGTCTGTGTATCC	935
PPI-Soy-FTA	CGCAGA--TATGGATAAACCAAGATTTAGATGATGATGAGAAAGGGCAACAACAAATTTA	954
Consensus	TGAAGAACCAGAGACTAACTTGGCCAATTTGGTGTGTACATTCTGTGTCTGTGTATCC	850
	970 980 990 1000 1010 1020	
BnA-12	AATAAGAGCTAACTATTGGGC--ATGG--	822
At-FT-A	TATAAGAGCTAACTATTGGGC--ATGGAGGAAGCAAGATTACAGTGGCAGCAATTG	992
PPI-Soy-FTA	AATAAGAGCTAACTATTGGGC--ATGG--AAAGATATGCAAAAT	1014
Consensus	AATAAGAGCTAACTATTGGGC--ATGG--AAAGATATGCAAAAT	889
	1030 1040 1050 1060 1070 1080	
BnA-12	-----	822
At-FT-A	AATATGTGACGCGCCCAAAATCACAATTGAAAAAGACTTGATTATTAGTTTTTACGTAATT	1052
PPI-Soy-FTA	TGGATTGTGGCGCAAGAGCAGACTTCCT	1041
Consensus	AT T G C G C A A C T	900
	1090 1100 1110 1120 1130 1140	
BnA-12	-----	822
At-FT-A	AACTGCTTATTTATGAATCACATGTTTCATGTTAACATGTATCAAAACAATCTTGATTCT	1112
PPI-Soy-FTA	-----	1041
Consensus	-----	900
	1150 1160 1170	
BnA-12	-----	822 (SEQ ID NO:12)
At-FT-A	CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1143 (SEQ ID NO:7)
PPI-Soy-FTA	-----	1041 (SEQ ID NO:37)
Consensus	-----	900 (SEQ ID NO:95)

Table 10H ClustalW Nucleic Acid Analysis of FT Beta

	10 20 30 40 50 60	
PPI-BnFTb	-----	1
eral	-----	1
PPI-Soy-FTB	-----	1
PPI-Corn-FTB	GGCGGATCCCGACCTACCGAGGCTCACGGTGACGAGGTGGAGCAGATGAAGGTGGAGGC	60
Consensus	-----	1
	70 80 90 100 110 120	
PPI-BnFTb	-----	1
eral	-----	1
PPI-Soy-FTB	-----GCCACCATTCTCGCAACGCCCAACCCCTCAT	32
PPI-Corn-FTB	CAGGGTTGGCGACATCTACCGCTCCCTCTTCGGGGCCGCGCCCAACACGAAATCCATCAT	120
Consensus	-----	1
	130 140 150 160 170 180	
PPI-BnFTb	-----	1
eral	-ATGGAGATTTCAGCGAGATAAGCAATTGGATTATCTGATGAAAGGCTTAAGGCAGCTTGG	59
PPI-Soy-FTB	GTTGGAGCTTCAACCGGATAATCACATGCAGTATCTCTCAAAGGCCTTCGCCATCTCAG	92
PPI-Corn-FTB	GCTAGAGCTGTGGCGTGATCAGCATATCGAGTATCTGACGCCTGGGCTGAGGCATATGGG	180
Consensus	T G A G T C G G A T A C A T A T A T T G G T G C A T G	27
	190 200 210 220 230 240	
PPI-BnFTb	-----	26
eral	TCCGCAGTTTCTTCCTTAGATGCTAATCGACCTTGGCTTGTGACTGGATTCTTCATTC	119
PPI-Soy-FTB	TTCCGCATTTTCCGTTTTCGACGCTAATCGACCTGGCTGTGCTACTGGATCTTCCATTC	152
PPI-Corn-FTB	ACCAGCCTTTTCATGTTCTAGATGCCAATCGCCTTGGCTATGCTACTGGATGGTTTCATTC	240
Consensus	C T T T T G A G C A A T C G C C T T G G C T T G T A C T G G A T T T C A T T C	65
	250 260 270 280 290 300	
	-----	

PPI-BnFTb	AATTGCTTTGCTTGGGAGTCTGTGGATGATGACTTAAAAAATGCAATCGATTTTCT	86
eral	AATAGCTTTGCTTGGGAGACTGTGGATGATGAATTAGAAAGCAATGCCATTGACTTCT	179
PPI-Soy-FTB	CATTGCTTTGTTGGGAGAAATCCGTGATGATGAACTCGAACATAACGGTATCGATTTTCT	212
PPI-Corn-FTB	ACTTGCTTTGCTGCGATGAAGCACTTGATGATGATCTTGAAGATGATATCATAGACTTCTT	300
Consensus	AATTGCTTTGCT GG GA C GT GATGATGA T GAAAA AATGC AT GA TT CT	111
		310 320 330 340 350 360
PPI-BnFTb	TGGACGTTGCCAGGCTTCTGATGGTGGATATGGTGGTGGTCTGGCCAACTTCCACATCT	146
eral	TGGACGCTGCCAGGGCTCTGAAGGTGGATACGGTGGTGGTCTGGCCAACTTCCACATCT	239
PPI-Soy-FTB	TAACGCTTGCCAGGATCGGAATGGTGGATATGCCGGGGACCGGCCAGATGCCTCATAT	272
PPI-Corn-FTB	AGCTCGATCTCAGGATAAAGATGGTGGATATAGTGGTGGACCTGCACATTGCCTCACT	360
Consensus	TG CG TGCCAGG T C GATGGTGGATATGGTGGTGG CCTGGCCA T CC CATCT	160
		370 380 390 400 410 420
PPI-BnFTb	TGCAACAACTTATGCTGCAGTGAATACACTTGTACTTTAGGAGGTGAAGAAAGCCCTTCTC	206
eral	TGCAACTACTTATGCTGCAGTGAATACACTTGTACTTTAGGAGGTGAAGAAAGCCCTTCTC	299
PPI-Soy-FTB	TGCACAACTTATGCTGCCTGTAAATACACTTATTACTTTGGTGGTGAAGAAAGCCCTGGC	332
PPI-Corn-FTB	AGCTACGACTTATGCTGCCTGTAAATACACTTGTGACAAATAGGGAGCGAAAGAGGATTGTC	420
Consensus	TGC AC ACTTATGCTGC GT AAT CACTTGTACTTTAGG GGTGA AAAGCC T TC	211
		430 440 450 460 470 480
PPI-BnFTb	TTCAATTAACAGAGAACAAATGGCTTGTTCITAAACCGAATGAAGGATACAAATGGAGG	266
eral	TTCAATTAATAGAGAAAAATGTCTTGTTCITAAACCGGATGAAGGATACAAATGGAGG	359
PPI-Soy-FTB	ATCAATTAATAGAGATAAACTGTATCGCTTCTGCGCGGATGAAGCAACCAATGGTGG	392
PPI-Corn-FTB	ATCAATCAATAGGGGCAACCTGTACAAATTTATGCTGCAGATGAAGATGTATCAGGTGC	480
Consensus	TCAATTAATAGAGA AAA TGT T GTTTT T G CGGATGAAGGAT CAA TGG GG	259
		490 500 510 520 530 540
PPI-BnFTb	TTTCAGGATGCATATATGCGGAGAAATAGATGTCGAGGCCGTGCTACACTGCCATTTTCA	326
eral	TTTCAGGATGCATGATATGCGGAGAAATGATGTTCCGTGCATGCTACACTGCATTTCCGT	419
PPI-Soy-FTB	ATTTCAGGATGCATGATGAAGGTGAAATGATGTTCCGAGCTTGCTACACTGCCATTTCCGT	452
PPI-Corn-FTB	TTTCAGATGTCATGATGGTGGGAAATGATGTCCTGTGCTTCTACACCGCTATATCCGT	540
Consensus	TTTCAGGATGCATGAT GG GAAATGATGT CG GC TGCTACACTGC ATTTCCGT	311
		550 560 570 580 590 600
PPI-BnFTb	TGCAAGCATCCTGAACATTGTGGATGATGAACCTACCCGCGGCTTAGGAGATTACATTTT	386
eral	TGCAAGCATCCTGAATATTATGGATGATGAACCTACCCAGGGCTTAGGAGATTACATCTT	479
PPI-Soy-FTB	TGCAAGTGTATTGAACATTTTGGATGATGAAGCTGATCCGAATGTTGGAGACTACATTAT	512
PPI-Corn-FTB	TGCCAGCCTTCTGAATATTCTTGATTTTAAACTGGCAAAAGGTCTAGGCAGCTACATAGC	600
Consensus	TGCAAGC T TGAA ATT TGGATGATGAAC T ACCCA GG TAGGAGA TACAT T	359
		610 620 630 640 650 660
PPI-BnFTb	GAGTTGCCAAACTTATGAAGGTGGCATTCGAGGGGAACCTGGCTCCGAAGCTCATGGTGG	446
eral	GAGTTGCCAAACTTATGAAGGTGGCATTCGAGGGGAACCTGGCTCCGAAGCTCATGGTGG	539
PPI-Soy-FTB	AAGCTGTCAAACATATGAAGGTGGCATTCGCTGGTGAAGCTGCTGAGCTCATGGTGG	572
PPI-Corn-FTB	AAGATGTCAAACCTTATGAAGGTGGTATTGCTGGGAGCCTTATGCTGAAGCATGGTGG	660
Consensus	AG TG CAAACTTATGAAGGTGGCATTC GGGGA CCTGG TC GAAGCTCATGGTGG	411
		670 680 690 700 710 720
PPI-BnFTb	GTACACCTACTGTGGGTTGGCTACTATGATTTAATCAATGAAGTCGACCGCTTGAATTT	506
eral	GTATACCTACTGTGGTTTGGCTCTATGATTTAATCAATGAGGTGCGACCGTTTGAATTT	599
PPI-Soy-FTB	GTACACCTTTGTGGATTAGCTACAATGATTCTGATTTGGTGAGGTAAATCACTTGCATCT	632
PPI-Corn-FTB	GTATACATTCTGTGGATTGGCTCCTTTGATCCTGCTTAATGAGGCAGAGAAAGTTGACTT	720
Consensus	GTA AC T CTGTGG TTGGCT CTATGATT T AT AATGAGGT GA C TTG ATTT	458
		730 740 750 760 770 780
PPI-BnFTb	GGATTCGTTAATGAATTGGGTTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAG	566
eral	GGATTCATTAAATGAATTGGGCTGTACATCGACAAGGAGTAGAAATGGGATTCCAAGGTAG	659
PPI-Soy-FTB	GCCTCGATTACTTCACTGGGTGGTATTCGACAAGGTAAGGAATGTGGATTCCAAGGTAG	692
PPI-Corn-FTB	GCCTAGTTTCAATTGGCTGGGTGCCTTTTCGTTCAAGGAGTGAATGCGGATTCCAAGGACG	780
Consensus	G T TTAAT A TGGGT GTA TCGACAAGGAGT GAA GGATT CAAGG AG	501
		790 800 810 820 830 840
PPI-BnFTb	GACGAACAAATTGGTTCGACGGTTGCTACACCTTTTGGCAGGAGGCCCCTGTGTTCTACT	626
eral	GACGAACAAATTGGTTCGATGGTTGCTACACATTTTGGCAGGAGGCCCCTGTGTTCTACT	719
PPI-Soy-FTB	AACAAATAAATGGTGGATGGATGCTATTCTTTTGGCAGGAGGCTGCTGTGCTCTATT	752
PPI-Corn-FTB	AACATAAATTGGTTGATGGTTGCTACTCTTTTGGCAGGAGGTTGCCATTGCTTTTAC	840
Consensus	AC AA AAATTGGT GATGGTTGCTAC C TTTTGGCAGG AGG C TG TCTA T	547

	850	860	870	880	890	900	
PPI-BnFTb	ACAGCGATT	TTTTCATCCCAG	GAT-ATGGCACCTCAT	GGATCATCA	TCACATATGT	CAC	685
eral	ACAAAGATTATAT	TCAACCAATGAT	CATGACGT-TCATGGATCAT	CA--CATATAT	CAG		775
PPI-Soy-FTB	GCAAAGATTATCT	TCTATTATCAAC-AAACAGATGGA	AGACATCA-C-----	AGATT			805
PPI-Corn-FTB	ACAAAAGTTAAT	TACGATTGTTGAT-AAAGCAA-----					871
Consensus	ACAAAGATTAT	TTC A	GAT-A G	A G	CATCA-		574
	910	920	930	940	950	960	
PPI-BnFTb	AAGGCACAGATGA	AGATCACCAGGAACAT	GGTCATGATGAAGAT	GATCCTCAAGACAGT	G		745
eral	AAGGCACAAATGA	AGAAACAT-----CATGCTCATGAT	GAAGATGACCTTGAAGACAGT	G			829
PPI-Soy-FTB	TTGCCGTATCTTAT	TCTATCTCAAGCAAAAGAAAGTT	GGATGGAACCTCTAGTCAT	GCAA			865
PPI-Corn-FTB	TTGAGGT-CCTCGTAT	TCTG---CAAAAGGCCATCAGGAGAGGAT	GCTG--CA-GCAC				924
Consensus	G G A T A G C TG	A A S CAT A GA G	CCTG	A			598
	970	980	990	1000	1010	1020	
PPI-BnFTb	ATGAAGATGAT---	TCTGATGAGGATACCGAT	GAAGATTCAGGGAAT	GCTCACCAGTT			802
eral	ATGATGATGATGAT	TCTGATGAGGACACGAT	GAAGATTCAGTGAAT	GCTCACAAGATCC			889
PPI-Soy-FTB	CATGCCGTGCTGAG	CATGAAGCACCAGTGAATCCAGTTCAT	CTGATTTTAAAAATATT	G			925
PPI-Corn-FTB	CATTTCTATA-TGG	TGCACCCGCAATAAGTCTTCCTCTGCTG	CACTATGCCAAGTT	G			983
Consensus	G	ATG TG T TGA S G A A	GAT	TTGAG S AT T A AA TT			629
	1030	1040	1050	1060	1070	1080	
PPI-BnFTb	ATCATACCTCTAC	CTTACATTGACAGGAGAAT	TCAACCTGTTTTGATAGCCT	CGGCTTGC			862
eral	ATCATACATCCAC	CTTACATTGACAGGAGAAT	TCAACCTGTTTTGATAGCCT	CGGCTTGC			949
PPI-Soy-FTB	CCTATAAAATTTAT	TAAATGAGTGGAGAGCACAAGAACCACT	TTTTTCACTATTGCTTTAC				985
PPI-Corn-FTB	GATTTGATTTTATA	CAACAGAGCAACCAAT	TGGGCCACTCTTCCATAACAT	TGCCCTGC			1043
Consensus	ATA T TA A	CAG AAT AACC	TTTTT ATAGC T G	CTTGC			663
	1090	1100	1110	1120	1130	1140	
PPI-BnFTb	AAAGATATCTGCT	CTTGCTCTCAGGT	TGCTGATGGTGGATT	CAGAGACAAGCT	GAAGA		922
eral	AGAGATATCTACT	CTTGCTCTAAGATCCGTCAGGTGGATT	CAGAGACAAGCCGAGGA				1009
PPI-Soy-FTB	AGCAATATATTCT	CTTATGCTGACAGGAGCAAGAGGGTGGAT	CTCAGAGACAAACCGGCTA				1045
PPI-Corn-FTB	AACAATACATCTCT	ACTTTGTTCTCAGGTACTAGAGGGAGGCTT	CAGGATTAAGCCCTGAA				1103
Consensus	A	ATAT T CTCTT TG TCTCAGGT C	GA GGTGGATT	AGAGACAAGCCG G A			709
	1150	1160	1170	1180	1190	1200	
PPI-BnFTb	AACCCCGTGACTT	CTACACACATGTTACTGCCTAAGCGCT	CTTTCCGTGGCTCAACAG				982
eral	AACCCCGTGACTT	CTACACACATGTTACTGCCTAAGCGCT	CTTTCCGTGGCTCAGCAG				1069
PPI-Soy-FTB	AACGTAGAGATCAT	TATCACACATGTTACTGTTTAAAGTGA	CTCTCATTTGTCAGTATA				1105
PPI-Corn-FTB	AGAACAGAGATCA	TATCATTCATGCTACTGCCTCAGTGGCT	CCGAGTTAGCCAGTACA				1163
Consensus	AAC C G GA	CTA CACACATGTTACTGCCT	AG GG CT TC GTG	CAG AC			752
	1210	1220	1230	1240	1250	1260	
PPI-BnFTb	CTTGGTCAAAAGAG	CAGGACACTCCCTCGTTT	GACTGCTGACATTTTGGTGGCT	TACGAAA			1042
eral	CTTGGTTAAAAGAG	CAGGACACTCCCTCGTTT	GACTGCTGACATTTTGGTGGCT	TACTGAA			1129
PPI-Soy-FTB	GTTGGTCAAAAGC	AGCCAGATTCTCCACCAC					1135
PPI-Corn-FTB	GTGCCATGACTGA	TACTGTTGTCGGGATTACCTCAGCAT	TGTGCTTGGACCGTACT	CTA			1223
Consensus	TTGGT AAA GAC	GA CTCC CC TT CTC	A T T GG	TAC S A			786
	1270	1280	1290	1300	1310	1320	
PPI-BnFTb	ACCACCTTGAACCT	GTTCACCTCCTCCACAACAT	TGTCTTGGATCGGTATTATGAAGCTT				1102
eral	ATCTCCTTGAACCT	GTTCACCTCCTCCACAACAT	TGTCTTGGATCGGTATTATGAAGCTA				1189
PPI-Soy-FTB							1135
PPI-Corn-FTB	ATTTGCTGGAGCCA	ATCCATCC-----					1245
Consensus	A	CT GA CC T CA C					797
	1330	1340	1350	1360	1370	1380	
PPI-BnFTb	CTAGATTT						1110
eral	TCGAGTTCTTCTT	TAAAGCAGCATGACCCGTTGTTGCTAAT	GTATGGGAAACCCCAAACA				1249
PPI-Soy-FTB							1135
PPI-Corn-FTB							1245
Consensus							797
	1390	1400	1410	1420			
PPI-BnFTb					1110 (SEQ ID NO:14)		
eral	TAAGAGTTTCCGTAGT	GTTGTAACCTTGTAAAGATTTCAAAG			1290 (SEQ ID NO:1)		

PPI-Soy-FTB	-----	1135	(SEQ ID NO:40)
PPI-Corn-FTB	-----	1245	(SEQ ID NO:43)
Consensus	-----	797	(SEQ ID NO:97)

### Example 13: Vector constructs for Transformation

The FTA or FTB sequences have been used to produce constructs suitable for transformation into plants and under the control of appropriate regulatory sequences. The gene sequences were in either the sense orientation for over-expression or the antisense orientation for down-regulation. Portions of these sequences have been used to construct a double-stranded-RNA-inhibition (dsRNAi) construct. A sequence of preferably not less than 21 nt was cloned as an inverse repeat separated by a linker that when expressed results in down-regulation of the target gene. Double antisense (DA) vectors have been created in which a direct repeat of an antisense sequence is separated by a spacer sequence such as GUS. Promoters have been used for constitutive expression such as the 35S CaMV promoter, the MuA *Zea mays* promoter or inducible by specific environmental or cellular cues such as the ABA levels or drought conditions which induce expression of the RD29A promoter. Alternatively, tissue or organelle specific promoters such as the HIC or CUT1 promoter can be used. Such constructs have been transformed into *Arabidopsis thaliana*, *Brassica*, *Zea mays*, *Glycine max*. Other species can be transformed as desired. Each species to be transformed may make use of specific regulatory sequences as appropriate for those particular species. Transformed plants have been selected and their phenotypic properties analyzed. The transgenic plants were assessed for characteristics such as increased tolerance to drought, altered biomass accumulation, yield, nutritional requirements such as minerals or micro-nutrients, biotic stress such as fungal, bacterial, or other such pathogen infection or attack or any other such physical or biochemical characteristic.

### Example 14: Plant Transformation

*Arabidopsis thaliana* transgenic plants were made by flower dipping method into an *Agrobacterium* culture. Wild type plants were grown under standard conditions until they began flowering. The plant was inverted for 2 min into a solution of *Agrobacterium* culture. Plants were then bagged for two days to maintain humidity and then uncovered to continue growth and seed development. Mature seed was bulk harvested.

Transformed T1 plants were selected by germination and growth on MS plates containing 50 µg/ml kanamycin. Green, kanamycin resistant seedlings were identified after 2 weeks growth and transplanted to soil. Plants were bagged to ensure self fertilization and the T2

seed of each plant harvested separately. During growth of T1 plants leaf samples were harvested, DNA extracted and Southern analysis performed.

T2 seeds were analyzed for Kan<sup>R</sup> segregation. From those lines that showed a 3:1 resistant phenotype surviving T2 plants were grown, bagged during seed set, and T3 seed harvested from each line. T3 seed was again used for Kan<sup>R</sup> segregation analysis and those lines showing 100% Kan<sup>R</sup> phenotype were selected as homozygous lines. Further analysis was done using T3 seed.

Transgenic *Brassica napus* plants were produced using *Agrobacterium* mediated transformation of cotyledon petiole tissue. Seeds were sterilized as follows. Seeds were wetted with 95% ethanol for a short period of time such as 15 seconds. Approximately 30 ml of sterilizing solution I was added (70% Javex , 100µl Tween20) and left for approximately 15 minutes. Solution I was removed and replaced with 30 ml of solution II (0.25% mercuric chloride, 100µl Tween20) and incubated for about 10 minutes. Seeds were rinsed with at least 500 ml double distilled sterile water and stored in a sterile dish. Seeds were germinated on plates of 1/2 MS medium, pH 5.8, supplemented with 1% sucrose and 0.7% agar. Fully expanded cotyledons were harvested and placed on Medium I (Murashige minimal organics (MMO), 3% sucrose, 4.5 mg/L benzyl adenine (BA), 0.7% phytoagar, pH5.8). An *Agrobacterium* culture containing the nucleic acid construct of interest was grown for 2 days in AB Minimal media. The cotyledon explants were dipped such that only the cut portion of the petiole is contacted by the *Agrobacterium* solution. The explants were then embedded in Medium I and maintained for 5 days at 24°C, with 16,8 hr light dark cycles. Explants were transferred to Medium II (Medium I, 300 mg/L timentin,) for a further 7 days and then to Medium III (Medium II, 20 mg/L kanamycin). Any root or shoot tissue which had developed at this time was dissected away. Transfer explants to fresh plates of Medium III after 14 -21 days. When regenerated shoot tissue developed the regenerated tissue was transferred to Medium IV (MMO, 3% sucrose, 1.0% phytoagar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin). Once healthy shoot tissue developed shoot tissue dissected from any callus tissue was dipped in 10X IBA and transferred to Medium V (Murashige and Skooge (MS), 3% sucrose, 0.2 mg/L indole butyric acid (IBA), 0.7% agar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin) for rooting. Healthy plantlets were transferred to soil.

Transgenic *Glycine max*, *Zea maize* and cotton can be produced using *Agrobacterium*-based methods which are known to one of skill in the art. Alternatively one can use a particle or non-particle biolistic bombardment transformation method. An example of non-particle biolistic

transformation is given in U.S. Patent Application 20010026941. Viable plants are propagated and homozygous lines are generated. Plants are tested for the presence of drought tolerance, physiological and biochemical phenotypes as described elsewhere.

The following table identifies the constructs and the species which they have been transformed.

**Table 11.**

SEQ ID NO:	SEQ	Species Transformed	
SEQ ID NO:10	pBI121-35S-anti-AtFTA	Arabidopsis thaliana	
SEQ ID NO:46	pBI121-35S-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:47	pBI121-rd29A-anti-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:48	pBI121-35S-DA-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:49	pBI121-RD29A-DA-AtFTA	Arabidopsis thaliana	Brassica napus
SEQ ID NO:50	MuA-anti-GmFTA		Glycine max
SEQ ID NO:51	RD29A-anti-GmFTA		Glycine max
SEQ ID NO:52	MuA-HP-GmFTA-Nos-Term		Glycine max
SEQ ID NO:53	RD29AP-HP-GmFTA-Nos-Term		Glycine max
SEQ ID NO:54	pBI121-35S-Anti-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:55	pBI121-RD29AP-Anti-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:56	pBI121-35S-HP-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:57	pBI121-RD29AP-HP-AtFTB	Arabidopsis thaliana	Brassica napus
SEQ ID NO:58	pBI121-35S-AtFTB	Arabidopsis thaliana	
SEQ ID NO:59	MuA-anti-GmFTB-Nos-Term		Glycine max
SEQ ID NO:60	RD29AP-anti-GmFTB-Nos-Term		Glycine max
SEQ ID NO:61	MuA-HP-GmFTB-Nos-Term		Glycine max
SEQ ID NO:62	RD29AP-HP-GmFTB-Nos-Term		Glycine max
SEQ ID NO:63	MuA-anti-Zea maizeFTB-Nos-Term		Zea maiz

SEQ ID MuA-HP-Zea maizeFTB-Nos-  
NO:64 Term

e  
Zea  
maiz  
e

Non-limiting examples of vector constructs suitable for plant transformation are given in  
SEQ ID NO: 10, 46-64.

SEQ ID NO:10

gtttaccgcgccaataatatcctgtcaaacactgatagtttaaacgaaggcggaacgacaatctgatcatgagcgg  
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caacgttgaaggagccactcagccgcgggtttctggagtttaatgagctaagcacatacgtcagaaccattattgc  
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cagacaatcggtctgtctgatgcgcgctgttccggctgtcagcgcaggggcgcccggttcttttgtcaagaccga  
cctgtccggtgcctgaatgaactgcaggacgagcgcggctatcgtggctggccacgacgggcgttccttgcg  
cagctgtgctcgacgttgtcactgaagcgggaagggaactggctgtctattgggcgaagtgcggggcaggatctcctg  
tcatctcaccttgctcctgcccagaaagtatccatcatggctgatgcaatgcggcggtgcatacgttgcacggc  
tacctgcccattcgaccaccaagcgaacatcgcatcgagcgcagcactcggatggaagccggtcttgctgcgac  
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tgaatcaaaaggccatggagtcaaaagattcaaatagaggacctaacagaactcgccgttaaagactggcgaacagttca  
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cggaattgttaaagacgtcagcttcaaggagctcgtgacagtaatcgagctcatcttcccatcctcctaagcccggt  
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 tctccctgcaacatcaggacccagtttctctgcaacccatcgccgatgatgccacagttggtagttcttagagttat  
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 tgccacactgtgtagttgccggagtttaagaggagggttcttccgtgagtcgtagtcgcgaggagatcgctcgtc  
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 gataaattatcgcgcgccgggtgtcatctatgttactagatcggggaattcactggccgtcggttttacaacgtcgtagt  
 gggaaaaccctggogttacccaacttaatcgccctgcagcacatcccccttgcgcagctggcgtaatagcgaagag  
 gccgcacccgatcgcccttcccaacagttgcgcagcctgaatggcgcccgctccttctcgcttcttcccttcccttcc  
 tgcgcacgttcgcgggttctcccgctcaagctctaaatcgggggctccctttaggggtccgatttagtgctttacgg  
 cacctcgacccccaaaaaacttgatttgggtgatgggttcacgtagtgggccatcgccctgatagacgggttttgcgcc  
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 gcgtggaccgcttgctgcaactctctcagggccaggcggtgaagggcaatcagctgttgcccgctcactgggtgaaa  
 agaaaaaccaccccgatcattaaaaacgtccgcaatgtgttattaagtgtctaacgctcaatttgtttacaccac  
 aatatactctgcca

SEQ ID NO:10 is the nucleic acid sequence of pBI121-antisense-FTA vector construct used to transform *Arabidopsis* plants. *Italicized* sequences are the right and left border repeats (1-24, 5226-5230). *Underlined* sequence is the 35S promoter (2515-3318). *Bold* sequence is the anti-sense Farnesyl transferase alpha sequence (3334-4317).

#### SEQ ID NO:46

gtttaccgcgccaatataatcctgtcaaacactgatagtttaaaactgaaggcgggaaacgcacaatc  
 tgatcatgagcggagaattaagggagtcacgttatgacccccgcgcatgacgcgggacaagccg  
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**TGGCCAAGTTAGTCTCTGGTTCTTCATTAGCTAGAGCTCTCACTGAGTCTTTATGCTCGTTGGT**  
**TGGTCTCAGTCCATCACATAGAAGATCCAAAAGGGTGCTCAGAGCGAATCCATGGAAGCAATCT**  
**GTGCGGGATAGAACATTCAAACAGACTGAGGAAACACTTGGATCACTAATCCAGGATTCTTTGT**  
**CGTCTTTGTAAGAGCTTTTAGGTATCGCCATGAGCTCTCGTTTGCAGGATTGGTTAAATGGC**  
**TTTGATTGTGTAGCTTACTTCAGATTCTCTCATGGCTTCTAGGCCTCCCAACAAAGGAGATTGG**  
**GTGATGACATAATACCTCTGATTCCAGGCGGAATTGTTAAAGACGTCAGCTTCAAGGAGCTCGT**  
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(Underlined Seq: 35S promoter; Bold: AtFTA anti-sense sequence separated by GUS Seq.)

SEQ ID NO:49

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(Underlined Seq: RD29A promoter; Bold: AtFTA anti-sense sequence, separated by GUS Seq.)

SEQ ID NO:50

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAAACCTCTTGGGATGCCATTGCCCATCTAT  
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TATCTCCATTGACGTAAGGGATGACGCACAATCCAACATATCCATCGCAAGACCATTGCTCTATA  
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(Underlined MuA Promoter; Bold: *Glycine max* anti-FTA; lower case: NOS terminator Seq.)

SEQ ID NO:51

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(Underlined RD29A Promoter; Bold: *Glycine max* anti-*Glycine max* FTA; lower case: NOS terminator Seq.)

SEQ ID NO:52

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 TGAGGAGCACCGTGGAGTAAGAAGACGTTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG  
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(Underlined: *Glycine max* FTA Anti-Sense section; Bold: MuA Promoter; Italics: *Glycine max* FTA Sense section; lower case: NOS terminator Seq.)

SEQ ID NO:53

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(Bold lower case: RD29A Promoter; Underline, Upper case: Antisense GmFTA; Upper case: Sense GmFTA; lower case: NOS terminator)

#### SEQ ID NO:54

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ctggaacaacactcaaccctatctcggttatcttttgatttataagggattttgcccatttc  
ggaaccaccatcaaacaggattttcgccctgctggggcaaaccagcgtggaccgcttgctgcaac  
tctctcaggggccaggcgggtgaagggaatcagctgttgcccgctcactggtgaaaagaaaaac  
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(Underlined: 35S promoter; Bold: Sense AtFTB)

SEQ ID NO:59

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAACCTCTTGGGATGCCATTGCCCATCTAT  
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TGAGGAGCACCGTGGAGTAAGAAGACGTTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG  
TATCTCCATTGACGTAAGGGATGACGCACAATCCAATATCCATCGCAAGACCATTGCTCTATA  
TAAGAAAGTTAATATCATTTTCGAGTGGCCACGCTGAGCTCGTGGTGGAGAATCTGGGTGCTTTG  
ACCAACTATACTGGCACAATGAGAGTCCACTTAAACAGTAACATGTGTGATAATGATCTCTACG  
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TCGGAATACCACCCAGTCAACTAATCGAGGCAGATCCAAGTGATTAACCTCACCAATCAGAATC  
ATTGTAGCTAATCCACAAAAGGTGTACCCACCATGAGCCTCAGAACCAGGCTCACCAGCAATGC  
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AATGTTCAAAACACTTGCAACAGAAATGGCAGTGTAGCAAGCTCGAACATCAATTTACCTTCA  
TCATGCATCCTGAATCCACCATTGTTGCTTCATCCGCCGAGAAACCCATACAGTTTATCTC  
TATTAATTGATGCCAGGGATTTCTCACCACCCAAAGTAATAAGTGAATTAACAGCAGCATAAGT  
TGTGGCAATATGAGGCATCTGGCCTGGTCCCCCGGCATATCCACCATTCTGGATCCTGGCAACGG  
TTAAGAAAATCGATAGCGTTATCTTCGAGTTCATCATCGACGGATTCTCCCAACAAAGCAATGG  
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cgттаagcatgтаатааттаacatgтаатgcatgacgттаттатgagatgggtttttatgatt  
agagtccccgcaattatacattttaatacgcgatagaaaacaaaatatagcgcgcaaaactaggata  
aattatcgcgcgcggtgtcatctatgttactagatcggaattc  
 (Upper Case: MuA Promoter; Underlined: Antisense GmFTB; Lower case: NOS terminator)

SEQ ID NO:60

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 AAATTAAAAATGAATAGAAAAGGTGAATTAAGAGGAGAGAGGAGGTAAACATTTCTTCTATTT  
 TTTTCATATTTTCAGGATAAATTATTGTAAAAGTTTACAAGATTTCCATTTGACTAGTGTAATG  
 AGGAATATTCTCTAGTAAGATCATTTATTTTCATCTACTTCTTTTATCTTCTACCAGTAGAGGAAT  
 AAACAATATTTAGCTCCTTTGTAAATACAAATTAATTTTCTTCTTGACATCATTCATTTTAA  
 TTTTACGTATAAAATAAAAGATCATACTATTAGAACGATTAAGGAGAAATACAATTCGAATGA  
 GAAGGATGTGCCGTTTGTATAATAAACAGCCACACGACGTAAACGTAAATGACCACATGATG  
 GGCCAATAGACATGGACCGACTACTAATAATAGTAAGTTACATTTTAGGATGGAATAAATATCA  
 TACCGACATCAGTTTGTAAAGAAAAGGGAAAAAAGAAAAAATAAATAAAAGATATACTACCGA  
 CATGAGTTCCAAAAGCAAAAAAAGATCAAGCCGACACAGACACGCGTAGAGAGCAAAATGA  
 CTTTGACGTCACACCAGAAAACAGACGCTTCATACGTGTCCCTTTATCTCTCTCAGTCTCTCT  
 ATAAACTTAGTGAGACCTCCTCTGTTTTACTCACAAATATGCAAACCTAGAAAACAATCATCAG  
 GAATAAAGGGTTTGATTACTTCTATTGGAAAGGTGGTGGAGAATCTGGGTGCTTTGACCAACTA  
 TACTGGCACAATGAGAGTCCACTTAAACAGTAACATGTGTGATAATGATCTCTACGTTTACCCG  
 GTTTGTCTCTCAGTCCACCCTCTTGCTCCTGTGCACATAAGAGAATATATTGCTGTAAAGCAAT  
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 CATCCAACTTTCTTTTGCTTCAGATACATAAGATACCGCAAAAATCTGTGATGTCTCTCCAT  
 CTGTTTGTGTGATAATAGAAGATAATCTTTGCAATAGAGCAACAGCACCTCCCTGCCAAAAGGAA  
 TAGCATCCATCCACCAGTTTATTTGTTCTCCCCTGGAATCCACATTCTTACCTTGTCTGGAATA  
 CCACCCAGTCAACTAATCGAGGCAGATCCAAGTGATTAACCTCACCAATCAGAATCATTGTAGC  
 TAATCCACAAAAGGTGTACCCACCATGAGCCTCAGAACCAGGCTCACCAGCAATGCCACCTCA  
 TATGTTTGACAGCTTATAATGTAGTCTCCAACATTCTGGATCAGCTCATCATCCAAAATGTTCA  
 AAACACTTGCAACAGAAATGGCAGTGTAGCAAGCTCGAACATCAATTTACCTTCATCATGCAT  
 CCTGAATCCACCATTGTTGCTTCATCCGCCGAGAAACCCATACAGTTTATCTCTATTAAAT  
 GATGCCAGGGATTTCTCACCACCCAAAGTAATAAGTGAATTAACAGCAGCATAAGTTGTGGCAA  
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ATCGATAGCGTTATCTTCGAGTTCATCATCGACGGATTCTCCCAACAAAGCAATGGAGTGGAAG  
 ATCCAGTAGCAGAGCCAGGGTCGATTAGCGTCCAAAACGGAAAATGCGGAACTGAGATGGCGAA  
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 agattgaatcctggttgccggtcttgcatgattatcatataatttctggtgaattacggttaagc  
 atgtaataattaacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtccc  
 gcaattatacatttaatacgcgatagaaaacaaaatatagcgcgcaaactaggataaattatcg  
 cgcgcggtgtcatctatgttactagatcggaattc

(Upper Case: RD29A Promoter; Underlined: Antisense GmFTB; Lower case: NOS) terminator

SEQ ID NO:61

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAACCTCTTGGGATGCCATTGCCCATCTAT  
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 ACAGAAAGGCCATTGTTGAAGATACCTCTGCTGACATTGGTCCCCAAGTGGAAGCACCACCCCA  
 TGAGGAGCACCGTGGAGTAAGAAGACGTTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG  
 TATCTCCATTGACGTAAGGGATGACGCACAATCCAACATCCATCGCAAGACCATTGCTCTATA  
 TAAGAAAGTTAATATCATTTCGAGTGGCCACGCTGAGCTCGTGGTGGAGAATCTGGGTGCTTTG  
 ACCAACTATACTGGCACAATGAGAGTCCACTTAAACAGTAACATGTGTGATAATGATCTCTACG  
 TTTACCCGGTTTGTCTCTCAGTCCACCCTCTTGCTCCTGTGCACATAAGAGAATATATTGCTGT  
 AAAGCAATACTGTGAAAAAGTGGTTCTTGCTCTCCACTCATTAATAAATTTATAGGCAATAT  
 TTTTAAATCAGATGAACCTGGATTCACTGGTGCCTTCATGCTCACCACGGCATGTTGCATGACT  
 AGAGGTTCCATCCAAACTTTCTTTTGCTTCAGATACATAAGATAACCGCAAAAATCTGTGATGTC  
 TCTTCCATCTGTTTGTGATAATAGAAGATAATCTTTGCAATAGAGCAACAGCACCTCCCTGCC  
 AAAAGGAATAGCATCCATCCACCAGTTTATTTGTTCTCCCCTGGAATCCACATTCCTTACCTTG  
 TCGGAATACCACCCAGTCAACTAATCGAGGCAGATCCAAGTGATTAACCTCACCACATCAGAATC  
 ATTGTAGCTAATCCACAAAAGGTGTACCCACCATGAGCCTCAGAACCAGGCTCACCAGCAATGC  
 CACCCTCATATGTTTGACAGCTTATAATGTAGTCTCCAACATTCTGGATCAGCTCATCATCAA  
 AATGTTCAAAACACTTGCAACAGAAATGGCAGTGTAGCAAGCTCGAACATCAATTTACCTTCA  
 TCATGCATCCTGAATCCACCATTGGTTGCTTCATCCGCCGCAGAAACCATAACAGTTTATCTC  
 TATTAATTGATGCCAGGGATTTCTCACCACCCAAAGTAATAAGTGAATTAACAGCAGCATAAGT  
 TGTGGCAATATGAGGCATCTGGCCTGGTCCCCGGGCATATCCACCATTCCGATCCTGGCAACGG  
 TTAAGAAAATCGATAGCGTTATCTTCGAGTTCATCATCGACGGATTCTCCCAACAAAGCAATGG  
 AGTGGAAGATCCAGTAGCAGAGCCAGGGTCGATTAGCGTCCAAAACGGAAAATGCGGAACTGAG  
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 GGTGGTATTCCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAACAAATAAACTGGTGGATGGA  
 TGCTATTCTTTTGGCAGGGAGGTGCTGTTGCTCTATTGCAAAGATTATCTTCTATTATCAACA  
 AACAGATGGAAGAGACATCACAGATTTTTCGGGTATCTTATGTATCTGAAGCAAAAGAAAGTTT  
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 CAGTATAGTTGGTCAAAGCACCCAGATTCTCCACCACgagctcgaatttccccgatcggttcaaa  
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 tttctggtgaattacggttaagcatgtaataattaacatgtaatgcatgacgttatttatgagat  
 ggggtttttatgattagagtcccgcaattatacatttaatacgcgatagaaaacaaaatatagcg  
 cgcaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcggaattc

(Upper Case: MuA Promoter; Underlined: Antisense GmFTB; Bold: Sense GmFTB; Lower

case: NOS terminator)

SEQ ID NO:62



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 AAATTAATAATGAATAGAAAAGGTGAATTAAGAGGAGAGAGGAGGTAAACATTTTCTTCTATTT  
 TTTCATATTTTCAGGATAAATTATTGTAAAAGTTTTACAAGATTTCCATTTGACTAGTGTAATG  
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 CATGAGTTCCAAAAGCAAAAAAAGATCAAGCCGACACAGACACGCGTAGAGAGCAAAATGA  
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 ATAACTTAGTGAGACCCTCCTCTGTTTTACTCACAAATATGCAAACCTAGAAAACAATCATCAG  
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 GATGCCAGGGATTTCTCACCACCCAAAGTAATAAGTGAATTAACAGCAGCATAAGTTGTGGCAA  
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 TCCGACAAGGTAAGGAATGTGGATTCCAGGGGAGAAACAAATAAACTGGTGGATGGATGCTATT  
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 aataaagtttcttaagattgaatcctgttgccggtcttgcgatgattatcatataatttctggt  
 gaattacggttaagcatgtaataattaacatgtaatgcatgacgttatttatgagatgggtttt  
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 aggataaattatcgcgcgcggtgtcatctatgttactagatcggaattc  
 (Upper Case: RD29A Promoter; Underlined: Antisense GmFTB; Bold: Sense GmFTB; Lower  
 case: NOS terminator)

SEQ ID NO:63

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 TGAGGAGCACCGTGGAGTAAGAAGACGTTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG  
 TATCTCCATTGACGTAAGGGATGACGCACAATCCAATATCCATCGCAAGACCATTGCTCTATA  
 TAAGAAAGTTAATATCATTTTCGAGTGGCCACGCTGAGCTCGGATGGATTGGCTCCAGCAAATTA  
 GAGTACGGTCCAAGCACATGCTGAGGTAATGGGCACGAACCAGTATCAGTCATGGCACTGTACT  
 GGCTAACTGCGAGGCCACTGAGGCAGTAGCATGAATGATAGTGATCTCTGTTCTTTCCAGGCTT  
 ATCCCTCAAGCCTCCCTCTAGTACCTGAGAACAAAGTAGGATGTATTGTTGCAGGGCAATGTTA  
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 catctatggttactagatcgggaattc

(Upper Case: MuA Promoter; Underlined: Antisense *Zea maize*-FTB; Lower case: NOS terminator)

## SEQ ID NO:64

GAATTCAAATTTTTCGCCAGTTCTAAATATCCGGAAACCTCTTGGGATGCCATTGCCCATCTAT  
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 TGAGGAGCACCGTGGAGTAAGAAGACGTTTCGAGCCACGTCGAAAAAGCAAGTGTGTTGATGTAG  
 TATCTCCATTGACGTAAGGGATGACGCACAATCCAATATCCATCGCAAGACCATTGCTCTATA  
 TAAGAAAGTTAATATCATTTTCGAGTGGCCACGCTGAGCTCGGATGGATTGGCTCCAGCAAATTA  
 GAGTACGGTCCAAGCACATGCTGAGGTAATGGGCACGAACCAGTATCAGTCATGGCACTGTACT  
 GGCTAACTGCGAGGCCACTGAGGCAGTAGCATGAATGATAGTGATCTCTGTTCTTTCCAGGCTT  
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 TGGCCTTTTGCAGGAATACGAGGACCTCAATTGCTTATCAACAATCGTAATTAACCTTTTGTGTG  
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 CTTTTGCCAGTTTAAAATCAAGAATATTCAACAAGGCTGGCAACCGATATAGCGGTGTAGGAAGC  
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 ATAAAATTGTACAGGTTGCCCTATTGATTGATGACAATGCTCTTTCGCTCCCTATTGTCACAA



GTGTATTTACAGCAGCATAAGTCGTAGCTAGGTGAGGCAACTGTCCAGGTCCACCACTATATCC  
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 CCAACCCTGGCCTCCACCTTCATCTGCTCCACCTGCGTCACCGTGAGCCTCGGTAGGTCTGGGAT  
 CCGCCgga**tcc**GCTGGGGAGCCTTATGCTGAAGCACATGGTGGGTATACATTCTGTGGATTGGC  
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 aaacaaaatatagcgcgcaaacactaggataaattatcgcgcgcggtgtcatctatgttactagat  
 cggaaagcctt

(Upper Case: MuA Promoter; Underlined: Antisense *Zea maize*-FTB; Bold: Sense *Zea maize*-FTB; Lower case: NOS terminator)

#### Example 15: PCR Analysis of Putative Transgenic Plants

To verify that the putative transgenic plants carried the gene of interest PCR analysis was performed. Genomic DNA was isolated and PCR run according to standard protocols and conditions which are known to one of skill in the art. A typical reaction was performed in a volume of 25 µl and primer pairs used were dependent on the gene and promoter combination of the particular construct (Table 12).

Putative transgenic *Brassica napus* plants were screened using the primer combinations detailed in the table below. A representative gel showing PCR analysis results is shown in Figure 24 which represents transgenic plants carrying the pRD29A-anti-FTA construct. Transformants were confirmed in an analogous manner for each species and construct transformation done.

Table 12.

Construct Name	Primer Name	Primer Sequence (5'-3')
35S-antiFTA	SEQ ID NO:16	GCCGACAGTGGTCCCAAAGATGG
	SEQ ID NO:17	AAAGGATCCTCAAATTGCTGCCACTGTAAT
rd29A-antiFTA	SEQ ID NO:18	AAACCCGGGATGAATTTTCGACGAGAACGTG
	SEQ ID NO:19	GCAAGACCGGCAACAGGA
rd29B-antiFTA	SEQ ID NO:20	TTTAAGCTTGACAGAAACAGTCAGCGAGAC

	SEQ ID NO:17	AAACCCGGGATGAATTTTCGACGAGAACGTG
35S-DA-FTA	SEQ ID NO:21	GCTCTTCCTCCATGCCCA
	SEQ ID NO:19	GCAAGACCGGCAACAGGA
rd29A-DA-FTA	SEQ ID NO:22	TTTAAGCTTGGAGCCATAGATGCAATTCAA
	SEQ ID NO:23	CGGGCATTAGGAGGATGGGAA
35S-HP-FTB	SEQ ID NO:16	GCCGACAGTGGTCCCAAAGATGG
	SEQ ID NO:24	GTCCGGAATTCCTCCGGGTC
rd29A-HP-FTB	SEQ ID NO:22	TTTAAGCTTGGAGCCATAGATGCAATTCAA
	SEQ ID NO:24	GTCCGGAATTCCTCCGGGTC

**Example 16: Southern Analysis**

Genomic Southern analysis of anti-FTA transgenic *Arabidopsis thaliana*. The numbers indicate the line numbers. Five micrograms of genomic DNA of T1 plants was digested with *Hind*III (a unique site in the T-DNA plasmid) and separated in a 0.8% agarose gel. The NPTII coding region was used as the probe for radio-labeling. Figure 11 shows a typical result from Southern analysis indicating the presence of the transgene.

**Example 17: Northern blots of antisense FTA lines**

RNA was isolated from developing leaf tissue of five 35S-anti-FTA *Arabidopsis thaliana* lines (T3 plants). The blot was first probed with P<sup>32</sup> labeled, single-stranded sense transcript of FTA (Figure 3 panel A) which detects antisense transcript, then stripped and re-probed with the single-stranded anti-sense transcript of FTA (Figure 12 panel B) that detects the sense transcript. Figure 3 panel C shows the ethidium bromide stained gel for the blot. Approximately 5 µg of total RNA was loaded into each lane. Figure 3 indicates the accumulation of the transgene anti-sense transcript and a reduction in the sense transcript in transgenic plants.

**Example 18: Western blot antisense FTA lines with Anti-FT-α antibodies.**

The antibodies produced according to the methods of Example 27 were used to analyze protein extracts from transgenic plants on western blots. Lane 1 of Figure 13 is a molecular weight standard, lane 2 purified FTA protein, lanes 3-10 are protein extracts from the ERA1 mutant, wild type, and 4 lines of transgenic *Arabidopsis thaliana*. Figure 13 illustrates the reduction of detectable FTA protein in transgenic lines.

**Example 19: ABA sensitivity of transgenic seedlings.**

Seeds of wild type Columbia, era1-2 and T3 homozygous seeds of two antisense, drought tolerant lines of 35S-antisense-FTA were plated on minimum medium (1/2 MS) supplemented with no ABA (A), 0.3 µM (B), 0.5 µM (C) or 1.0 µM ABA (D). Plates were

chilled for 3 days in 4 °C in the dark, and incubated for 11 days at 22 °C with 24 hour continuous light. *era1* and transgenic lines were more inhibited in germination than wild type plants. Results are shown in Figure 14.

Twelve day old seedling phenotypes of wild type Columbia, *era1-2* and two drought tolerant 35S-antisense-FTA lines (9.9 & 21.2) in minimum medium without (A) or with (B) 1 µM ABA. Figure 15 shows the reduced root growth and development of *era1* and transgenic lines relative to wild type plants. The 35S-antisense-FTA lines show reduced root growth, similar to the *era1* mutant, in response to ABA.

A transgenic *Brassica napus* line carrying the 35S-antisense-FTA construct was assessed for ABA sensitivity. At about 10µm an effect was observed showing reduced seedling development and vigor at the cotyledon and first leaf stage, thereby indicating an increased sensitivity to ABA

ABA sensitivity is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the methods above. The ABA concentration used varies depending upon the species under examination.

#### **Example 20: Drought Experiment**

To assess the response of plants under water stress or drought one can expose plants to various situations. For example, the plant can be removed from soil or media and placed on paper towel for a period of time, such as 4 hours, then returned to a plate to continue growth and development. Survival and vigour can be assessed.

Alternatively one can impose a water stress in such a way as to more closely resemble a field situation by withholding water for a period of time, such as up to 6 days. Plants were grown five plants per four inch pot, in a replicated water-stress experiment. All pots were filled with equal amounts of homogeneous premixed and wetted soil. Growth conditions were 16 hour daylight (150-200 µmol/m<sup>2</sup>/s) at 22 °C and 70% relative humidity. On the day that the first flower opened drought treatment was initiated first by equalizing the soil water content in each pot on a weight basis and then cessation of watering. At the end of the water stress treatment plants were typically either harvested for biomass data or re-watered to complete the life cycle and determination of biomass and yield data. Physiological parameters have been assessed under stressed and optimal conditions, for example, shoot and root biomass accumulation, soil water content, water loss alone or as a function of parameters such as biomass, seed yield, and leaf number and leaf area. Figure 16 shows photographs of wild type Columbia (A) and four 35S-antisense-FTA transgenic *Arabidopsis thaliana* lines (B,C,D,E) after 8 days of water stress

treatment. The control plant is visibly stressed and less healthy. This experiment has been conducted on transgenic lines containing vectors described by SEQ ID NO: 10, 46-64.

Drought or water stress tolerance is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the described methods.

**Example 21: Analysis of Water Loss in *Arabidopsis thaliana* pRD29A-DA-FTA lines during drought stress**

Plants were grown 5 plants per 4 inch pot and 6 pots per line. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Pots were weighed daily and at the end of the 7 day drought treatment all plants were harvested for shoot fresh weight and dry weight determinations. Figure 10 shows the water loss on a per shoot dry weight basis at 4 days of water stress treatment. Of the 31 lines examined in this experiment 25 showed lower water loss relative to the Columbia wild type, 22 of which were statistically significant. All lines had been assessed for ABA sensitivity as described in Example 14, increased ABA sensitivity ( $ABA^S$ ) also correlated with a decreased water loss during drought treatment. Those lines determined to have wild type ABA sensitivity ( $ABA^{WT}$ ) were the same 6 lines (lines 2, 36, 69, 29, 24, 21) that did not show a reduced water loss compared to wild type.

The above experiment was repeated using two  $ABA^S$  lines, one  $ABA^{WT}$  line and a Columbia control. Plants were harvested after 2, 4 and 6 days of water stress treatment for shoot dry weight determinations.  $ABA^S$  transgenics had greater leaf and shoot biomass, greater soil water contents and lower water loss per shoot dry weight when compared to the  $ABA^{WT}$  or Columbia controls. Results were consistent at all three harvest stages.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has also been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar water stress tolerant trends observed. Soil water loss is assessed in all transgenic plants engineered to have reduced or increased FTA or FTB expression or activity by the described methods.

**Example 22: Analysis of Shoot Fresh Weight in *Arabidopsis thaliana* pRD29A-DA-FTA lines during drought stress**

Plants were grown 5 plants per 4 inch pot and 8 pots per line. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Plants were re-watered after 6 days drought treatment and allowed to recover for an additional 6 days. Plants were harvested and shoot fresh weights determined. Figure 20 shows the shoot fresh weights.

This experiment consisted of 25 transgenic lines, 2 of which are ABA<sup>WT</sup> (line 2 and 69) and a Columbia wild type control. All 23 ABA<sup>S</sup> transgenic lines had statistically significant greater shoot fresh weights, on average 44% greater.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

**Example 23: Analysis of seed yield in *Arabidopsis thaliana* pRD29A-DA-FTA lines during drought stress and under optimal conditions**

Plants were grown 1 plant per 4 inch pot. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Plants were re-watered after 6 days drought treatment and allowed to grow to maturity. The optimal group was not exposed to the drought treatment.

Yield analysis indicates that although drought treatment results in decreased yields, the transgenics do not suffer as severely as controls and maintain a productivity advantage (Figure 21) as shown previously in Experiment 22. Comparison of the yields produced by the ABA<sup>S</sup> transgenics versus the control plants show that a 15% greater yield was obtained under optimal conditions and a 20% increase under drought conditions. In the drought treatment group 8 of 9 transgenic lines showed greater yield than controls. Expression of yield of each line obtained under drought treatment as a percentage of its performance under optimum conditions indicates that 8 of 9 ABA<sup>S</sup> lines outperformed the control line while 4 of 9 out performed the ABA<sup>WT</sup> controls.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

**Example 24: Analysis of vegetative growth in *Arabidopsis thaliana* pRD29A-DA-FTA lines under optimum growth conditions**

Plants were grown 1 plant per 3 inch pot and 8 pots per line. Plants were harvested at three stages and fresh weights determined. Vegetative stage was defined as 14 day old seedlings, bolting stage as the appearance of first flower (19-21 day seedlings) and mid-flowering as 6 days from first flower. At each of the above stages respectively 7, 8 and 10 of the 10 ABA<sup>S</sup> transgenic lines tested showed statistically greater shoot fresh weight biomass than the control plants

(Figure 22). One Columbia line and an ABA<sup>WT</sup> (line 2) line were used as the control group. Additionally, there was a statistically significant trend for the transgenic lines to have an increased number of rosette leaves.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

**Example 25: Analysis of *Arabidopsis thaliana* pRD29A-DA-FTA lines under drought treatment and biotic stress**

Plants were grown 1 plant per 4 inch pot and 8 pots. When the plants had grown to the first flower stage drought treatment was initiated as described in Example 20. Plants were re-watered after 7 days drought treatment and allowed to grow to maturity. One Columbian control line (col) and one transgenic line were evaluated. Analysis of seed yield indicated less than normal yields, approximately 12% of expected optimal yield. It was determined that the soil used contained a fungal contaminant that was responsible for the reduced yields as the biotic stress could be negated by sterilization of the soil prior to use. This biotic stress was less severe in the transgenic line compared to the control which had a yield 22% of the transgenic line. In the drought treatment groups of plants the biotic stress was reduced however, transgenics outperformed controls by nearly 4.5 fold (Figure 23).

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

**Example 26: Analysis of *Arabidopsis thaliana* pRD29A-DA-FTA lines for Stomatal number**

The number of stomata on both the upper and lower surface of the leaf was assessed on two transgenic lines and a wild type Columbia control. Nail polish imprints were made of both upper and lower leaf surfaces of the fifth leaf, plants were at the early flowering stage. No differences in stoma density were observed.

The data shown in this example was obtained using transgenic plants carrying the pRD29A-DA-FTA construct. The experiment has been conducted on lines carrying variations of this construct such as 35S-DA-FTA, pRD29A-antisense-FTA or 35S-antisense-FTA, with similar trends observed.

**Example 27: Production of polyclonal antibodies against FT-A and FT-B**

The isolated *Arabidopsis thaliana* FT sequences were cloned into the *E. coli* expression vector derived from pET11D. To generate the Histidine tagged FT-B construct the *Arabidopsis thaliana* FT-B clone and pET vector were digested with *Bam*HI and ligated together. Restriction digests were performed to verify the orientation of the insert. To produce the FT-A construct the *Arabidopsis thaliana* FT-A clone and pET vector were digested with *Bam*HI and *Eco*RI and subsequently ligated together. The resultant plasmids directed the expression of fusion proteins containing 6 consecutive histidine residues at the N-termini of AtFTA and AtFTB. The fusion proteins were expressed in the bacterial host BL21(DE3) and purified using Hi-Trap chelating chromatography as described by the manufacturer (Pharmacia). The soluble fraction of the crude bacterial extract containing the His-FT fusion proteins were loaded to a Hi-Trap column (1.5 cm x 2.0 cm), and the proteins eluted with a 200 ml linear gradient of 0.0 to 0.3 M imidazole in column buffer (25 mM Tris-HCl, pH 7.5, 1 mM DTT). Fractions containing purified His-FT proteins were pooled, desalted and concentrated with a Centriprep-30 concentrator (Amicon). All purification steps were carried out at 4 °C. To generate an antibody, the purified fusion protein was further separated by SDS/PAGE and the Coomassie stained band corresponding to the fusion protein was excised. Protein was eluted from the gel slice by electroelution and then emulsified in Ribi adjuvant (Ribi Immunochem) to a final volume of 1 ml. His-AtFTA or His-AtFTB (250 µg) were injected into a 3 kg New Zealand rabbit on day 1 and booster injections given on day 21 and day 35 with 200 µg of the protein. High-titer antisera were obtained one week after the final injection. These antibodies were used in the western analysis of example 18, Figure 13.

**Example 28: Screening for related genes**

The transgenic plants of the invention can be used to identify genes which interact with the genes of the present invention. One can make use of the transgenic plants of the invention to screen for related genes, for example, suppressors, enhancers or modulators of gene expression or activity can be identified through genetic screening protocols. By way of example, a mutant library can be generated using the transgenic plants of the invention as the genetic background. Various methods are available and would be known to one of skill in the art. For example, chemical mutagens such as EMS can be used to induce point mutations in the genome, fast neutron irradiation of seeds can result in deletion mutations, T-DNA libraries can be produced that inactivate genes through insertional effects or activation tagging methods can be used to produce libraries with up-regulated genes. Analysis of these types of libraries can identify genes

which rescue or modulate the phenotypes observed in the transgenic plants of the present invention.

#### **Example 29: RT-PCR amplification and cloning of CaaX prenyl proteases**

Total RNA was isolated from leaf tissue of *Arabidopsis thaliana*, *Brassica napus* and *Glycine max*, using the Qiagen RNeasy kit and used as template to amplify the CPP genes by RT-PCR. Reaction conditions were as follows; 1X reaction buffer (10mM Tris-HCl pH 8.8, 1.5mM MgCl<sub>2</sub>, 50mM KCl), dNTP's at 200μM, 1pM AtCPP BamFW and AtCPP SmaRV primers, 2.5U. Pfu DNA polymerase, and template plus water to a final volume of 100μL. Reactions were run at 1 minute 94°C, 1 minute 60°C, 1 minute 72°C, for 30 cycles. Primers used to PCR amplify *Arabidopsis* and *Brassica* sequences were those identified by SEQ ID NO:101 and SEQ ID NO:102. Primers used to PCR amplify the *Glycine* sequence were those identified by SEQ ID NO:149 and SEQ ID NO:150. PCR products were separated from the RT-PCR reaction mixture using the Qiagen PCR column spin kit and ligated into the prepared cloning vector, pBluescript KS+. The vector had been prepared by digestion with *EcoRV* and treated with *Taq* polymerase in the presence of dTTP to produce a 3' overhang suitable for ligation with the PCR products. The ligation products were transformed into *E. coli* DH5α cells, positive colonies selected and the resulting inserts sequenced. The above methodology is applicable to obtain homologous sequences and may require alternative primers.

**Table 13.**

AtCPP BamFW: (SEQ ID NO:101)	5'-AAAGGATCCATGGCGATTCCTTTCATGG-3'
AtCPP SmaRV: (SEQ ID NO:102)	5'-AAACCCGGGTAAATCTGTCTTCTTGTCTTCTCCA-3' (SEQ ID NO:102)
GmCPP SmaFW: (SEQ ID NO:149)	5'-AAACCCGGGATGGCGTTTCCCTACATGGAAGCC -3'
GmCPP SacRV: (SEQ ID NO:150)	5'-AAAGAGCTCTTAGTCTTCCTTCTTATCCGGTTCG -3' (SEQ ID NO:150)

#### **Example 30: Vector Construction**

Construction of the pBI121-AtCPP construct (SEQ ID NO: 99) was prepared as follows. The pBI121 vector was digested with *Bam*HI and *Sma*I. The AtCPP, 1.4 kb DNA fragment from



RT-PCR (SEQ ID NO: 97) was digested with *Bam*HI and *Sma*I and ligated into the pBI121 vector. The GUS sequence was then removed by digestion with *Sma*I and *Eco*LCRI and the vector ligated after purification of the vector from the GUS insert to produce the pBI121-AtCPP vector (Figure 25A). This construct was used to further generate constructs expressing the CPP gene from *Brassica* and *Glycine*. To produce the pBI121-BnCPP construct (SEQ ID NO:142) primer pairs identified by SEQ ID NO:101 and SEQ ID NO:102 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector. To produce the pBI121-GmCPP construct (SEQ ID NO:136) primer pairs identified by SEQ ID NO:149 and SEQ ID NO:150 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector.

Construction of the pBI121-antisense-AtCPP construct (SEQ ID NO:130). The antisense fragment was produced using PCR amplification with SEQ ID NO:97 as template and primers identified as SEQ ID NO:106 and SEQ ID NO:107, listed in Table 14. This fragment was digested with *Bam*HI and *Sma*I and used to replace the sense fragment of the pBI121-AtCPP construct (SEQ ID NO:99), to yield SEQ ID NO:130 (Figure 25B). This construct, SEQ ID NO:130, was used to further generate constructs expressing the antisense CPP gene from *Brassica* and *Glycine*. To produce the pBI121-antisense-BnCPP construct (SEQ ID NO:144) primer pairs identified by SEQ ID NO:151 and SEQ ID NO:152 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector. To produce the pBI121-antisense-GmCPP construct (SEQ ID NO:138) primer pairs identified by SEQ ID NO:153 and SEQ ID NO:154 are used to PCR amplify the appropriate fragment which is ligated into the prepared parent vector.

Construction of the pBI121-HP-AtCPP construct (SEQ ID NO:100). The cloning strategy involved truncating the GUS gene of pBI121 and flanking the GUS sequence with a AtCPP fragment in the antisense orientation upstream of the GUS and in the sense orientation on the downstream side of GUS. The pBI121 vector was digested with *Sma*I and *Sac*I, the GUS sequence and the vector fragments were purified from one another. The isolated GUS fragment was digested using *Eco*RV and the 1079 bp. blunt ended *Eco*RV/*Sac*I fragment isolated. This was ligated back into the digested parent vector at the *Sma*I/*Sac*I sites. This intermediate vector was used in the subsequent production of the hair-pin vectors. The AtCPP fragment to be used as the gene specific hair-pin sequence was isolated by PCR. Primers identified as SEQ ID NO:103 and SEQ ID NO:104, listed in Table 14, were used to generate a 596 bp fragment. Cloning of the sense orientation fragment was achieved by digesting the PCR AtCPP fragment

with *SacI* and ligation into the *SacI* site at the 3' end of GUS. To insert the same fragment upstream of GUS, the *BamHI* site was opened and the ends blunted with Klenow. The PCR amplified AtCPP fragment was digested with *EcoICRI*, which is an isoschizomer of *SacI* but leaves blunt ends, and ligated into the blunted *BamHI* site of the vector to yield the final construct (Figure 25C). The intermediate construct used to produce SEQ ID NO:100 above contained only the truncated GUS gene and no CPP sequences this intermediate vector was used to further generate constructs expressing hair-pin CPP gene constructs from *Brassica* and *Glycine*. To produce the pBI121-HP-BnCPP construct (SEQ ID NO:143) primer pairs identified by SEQ ID NO:153 and SEQ ID NO:154 are used to PCR amplify the sense fragment and primer pairs identified by SEQ ID NO:155 and SEQ ID NO:156 are used to PCR amplify the antisense fragment. These fragments are cloned into the prepared intermediate vector described above. To produce the pBI121-HP-GmCPP construct (SEQ ID NO:137) primer pairs identified by SEQ ID NO:157 and SEQ ID NO:158 are used to PCR amplify the sense fragment and primer pairs identified by SEQ ID NO:159 and SEQ ID NO:160 are used to PCR amplify the antisense fragment. These fragments are cloned into the prepared intermediate vector described above.

The above vector constructs were modified to place the genes under the control of alternative promoters, such as, but not limited to, the RD29A or MuA. This was accomplished by excising the 35S promoter sequence and replacing it with an appropriate promoter sequence. In this way SEQ ID NO's:134 and 135 were generated and SEQ ID NO's:133, 136-148 can be constructed.

**Table 14**

AtCPP-HP-SacFW	5'-CTGGAGCTCTTTTACCGAGGTTGGGCCTTGATCC-3'	(SEQ ID NO:103)
AtCPP-HP-SacRV	5'-ATTGAGCTCCCAATGTCCAAGCTCGTGTGCAATA-3'	(SEQ ID NO:104)
AtCPP-anti-SmaFW	5'-AAACCCGGGATGGCGATTCCTTTTCATGG-3'	(SEQ ID NO:106)
AtCPP-anti-BamRV	5'-AAAGGATCCTTAATCTGTCTTCTTGTCTTCTCCA-3'	(SEQ ID NO:107)
BnCPP-anti-SmaFW	5'-AAACCCGGGATGGCGATTCCTTTTCATGG -3'	(SEQ ID NO:151)
BnCPP-anti-BamRV	5'-AAAGGATCCTTAATCTGTCTTCTTGTCTTCTCC -3'	(SEQ ID NO:152)
BnCPP-HP-Sac-FW	5'- AAAGAGCTCTTCTACCAATGGTGGGACTCG -3'	(SEQ ID NO:153)
BnCPP-HP-Sac-RV	5'- AAAGAGCTCCCAGTGTCCCAGCTCGTGTG -3'	(SEQ ID NO:154)

BnCPP-HP-BamFW 5'- AAAGGATCCTTCTACCAATGGTGGGACTCG -3'  
(SEQ ID NO:155)

BnCPP-HP-XbaRV 5'- AAATCTAGACCAGTGTCCCAGCTCGTGTG -3'  
(SEQ ID NO:156)

GmCPP-HP-Sac-FW  
5'-GATGAGCTCACAAGATCAAGTCACAGCAATGCCT -3'  
(SEQ ID NO:157)

GmCPP-HP-Sac-RV 5'- AAAGAGCTCCCGGTTTCGTCCAGCGCGGCC -3'  
(SEQ ID NO:158)

GmCPP-HP-BamFW  
5'- GATGGATCCACAAGATCAAGTCACAGCAATGCCT -3'  
(SEQ ID NO:159)

GmCPP-HP-XbaRV 5'- CCTTCTAGACCGGTTTCGTCCAGCGCGGCC -3'  
(SEQ ID NO:160)

### Example 31: Sequence Analysis

#### *Arabidopsis thaliana* CPP (AtCPP)

A disclosed nucleic acid of 1275 nucleotides (SEQ ID NO:97) and also referred to as AtCPP, is shown in Table 15.

**Table 15A. AtCPP Nucleotide Sequence (SEQ ID NO:97).**

```

ATGGCGATTCTTTTCATGGAAACCGTCGTGGGTTTATGATAGTGATGTACATTTTTGAG
ACGTATTTGGATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACCTCTCCCGAAAACCTTG
GTTGGTGTAATTAGCCAAGAGAAGTTTGAGAAATCACGAGCATAACAGTCTTGACAAAAGC
TATTTTCACTTTGTTTCATGAGTTTGTAACATACTTATGGACTCTGCAATTTTGTTCTTT
GGGATCTTGCTTGGTTTTGGAAGATGTCTGGAGCTGTTTTACCGAGGTTGGGCCTTGAT
CCGGAGAATGAAATACTGCATACTCTTTCATTCTTGGCTGGTGTTATGACATGGTCACAG
ATCACTGATTTGCCATTTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTT
AACAAACAAACAATATGGATGTTTCATTAGGGACATGATCAAAGGAACATTCCTCTCTGTC
ATACTAGGCCCACCCATTGTTGCTGCGATAATTTTCATAGTCCAGAAAGGAGGTCTTAT
CTTGCCATCTATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATAC
CCGGTCTTGATAGCACCGCTCTTCAACAAATTCCTCTTCCAGATGGAGACCTCCGG
GAGAAGATTGAGAACTTGCTTCTTCCCTAAAGTTTCTTTGAAGAAGCTGTTTGTTGTC
GATGGATCTACAAGGTCAAGCCATAGCAATGCTTACATGTATGGTTTCTTTAAGAACAAA
AGGATTGTTCTTTATGATACGTTGATTGAGCAGTCAAGAATGAGGATGAAATTGTGGCG
GTTATTGCACACGAGCTTGGACATTGGAACCTGAATCACACTACATACTCGTTTCATTGCA
GTTCAAATCCTTGCTTCTTACAATTTGGAGGATACACTCTTCTCAGAACTCCACTGAT
CTCTTCAGGAGTTTCGGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTGAG
CACACTGTAATACCACTGCAACATCTAGTAAGCTTTGGCCTGAACCTCGTTAGTCGAGCG
TTTGAGTTTCAGGCTGATGCTTTTGCTGTGAAGCTTGACTATGCAAAGATCTTCGTCCT

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GCTCTAGTGAAACTACAGGAAGAGAACTTATCAACAATGAACACTGATCCATTGTACTCA GCTTATCACTACTCACATCCTCCTCTTGTGAAAGGCTTCGAGCCACTGATGGAGAAGAC <b>AAGAAGACAGATTAA</b>
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A disclosed CPP polypeptide (SEQ ID NO:98) encoded by SEQ ID NO:97 has 424 amino acid residues and is presented in Table 15B using the one-letter amino acid code.

**Table 15B. Encoded CPP protein sequence (SEQ ID NO:98).**

MAIPFMETVVGFMIVMYIFETYLDLRQLTALKLPTLPKTLVGVISQEKFEKSRAYSLDKS YFHFVHEFVTILMDSAILFFGILPFWKMSGAVLPRLGLDPENEILHTLSFLAGVMTWSQ ITDLPFSLYSTFVIESRHFNGKQTIWMFIRDMIKGTFLSVILGPPIVAIIIFIVQKGGPY LAIYLWAFMFILSLVMMTIYPVLIAPLFNKFPTPLPDGDLREKIEKLASSLKFPKKLFVV DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNHTTYSFIA VQILAFQLQGGYTLRNSTD LFRSFGFDTQPVLIIGLIIFQHTVIPLQHLVSFGLNLVSRA FEFQADAFVAVKLDYAKDLRPALVKLQEENLSTMNTDPLYSAYHYSHPPPLVERLRATDGED KKTD
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The present invention also includes a nucleic acid sequence complimentary to the *Arabidopsis thaliana* CaaX prenyl protease of SEQ ID NO:97. The disclosed complimentary sequence is shown as SEQ ID NO:115.

SEQ ID NO:115

TTAATCTGTCTTCTTGTCTTCTCCATCAGTGGCTCGAAGCCTTCAACAAGAGGAGGATGTGAG  
TAGTGATAAGCTGAGTACAATGGATCAGTGTTCATTGTTGATAAGTTCTCTTCTCTGTAGTTTCA  
CTAGAGCAGGACGAAGATCTTTTGCATAGTCAAGCTTCACAGCAAAAGCATCAGCCTGAAACTC  
AAACGCTCGACTAACGAGGTT CAGGCCAAAGCTTACTAGATGTTGCAGTGGTATTACAGTGTGC  
TGAAATATGATCAAACCAATGAGAACAGGCTGTGTATCAAATCCGAAACTCCTGAAGAGATCAG  
TGGAGTTTCTGAGAAGAGTGTATCCTCCAAATTGTAAGAAGGCAAGGATTTGAACTGCAATGAA  
CGAGTATGTAGTGTGATT CAGTTTCCAATGTCCAAGCTCGTGTGCAATAACCGCCACAATTTCA  
TCCTCATTTCTTGCACTGCTGAATCAACGTATCATAAAGAACAATCCTTTTGTCTTAAAGAAAC  
CATACATGTAAGCATTGCTATGGCTTGACCTGTAGATCCATCGACAACAAACAGCTTCTTCAA  
AGGAAACTTTAGGGAAGAAGCAAGTTTCTCAATCTTCTCCCGAGGTCTCCATCTGGAAGAGGA  
GTGAATTTGTTGAAGAGCGGTGCTATCAAGACCGGGTATATAGTCATCATCACTAGAGACAGGA  
TAAACATGAATGCCACAGATAGATGGCAAGATAAGGACCTCCTTTCTGGACTATGAAAATTAT  
CGCAGCAACAATGGGTGGGCCTAGTATGACAGAGAGGAATGTTCTTTGATCATGTCCCTAATG  
AACATCCATATTGTTTGTGTTGAACCCATGCCGAGACTCGATCACGAAAGTTGAGTACAAAG

AAAATGGCAAATCAGTGATCTGTGACCATGTCATAACACCAGCCAAGAATGAAAGAGTATGCAG  
TATTTTCATTCTCCGGATCAAGGCCAACCTCGGTAAAACAGCTCCAGACATCTTCCAAAACCAA  
GGCAAGATCCCAAAGAACAAAATTGCAGAGTCCATAAGTATAGTTACAAACTCATGAACAAAGT  
GAAAATAGCTTTTGTCAAGACTGTATGCTCGTGATTTCTCAAACCTTCTCTTGGCTAATTACACC  
AACCAAGGTTTTCGGGAGAGTTGGAAGCTTGAGAGCAGTGAGTTGCCTCAGATCCAAATACGTC  
TCAAAAATGTACATCACTATCATAAAACCCACGACGGTTTCCATGAAAGGAATCGCCAT

Due to the nature of the cloning strategy the sequence presented is not full length but is missing the 5' and 3' non-translated regions. The percent identities of the *Arabidopsis thaliana* nucleotide sequence and its encoded amino acid sequence to that of other CPP sequences as determined by ClustalW analysis are shown in Figure 26.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

#### ***Brassica napus* CPP (BnCPP)**

A disclosed nucleic acid of 1275 nucleotides (SEQ ID NO:109) and also referred to as BnCPP, is shown in Table 16.

**Table 16A. BnCPP Nucleotide Sequence (SEQ ID NO:109).**

ATGGCGATTCCCTTTCATGGAAACCGTCGTTGGTTTTATGATAGTGATGTACGTT
TTTGAGACGTATTTGGATCTGAGGCAACATACTGCTCTCAAGCTTCCCACTCTC
CCAAAGACTTTGGTTGGAGTCATTAGCCAAGAGAAGTTTGAGAAATCTCGAGCT
TACAGTCTTGACAAAAGCCATTTTCACTTTGTTTCATGAGTTTGTTACTATACTT
ATGGACTCTGCGATTCTGTTCTTTGGGATCTTGCCCTTGGTTTTGGAAGATATCT
GGCGGCTTTCTACCAATGGTGGGACTCGATCCAGAGAATGAAATCCTGCACACT
CTTTCATTCTTGGCTGGTCTTATGACATGGTCACAGATCACTGATTTGCCATTT
TCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTCAACAAACAAACA
ATATGGATGTTTCATTAGGGACATGATCAAAGGAATACTCCTCTCTGTCATACCT
GCCCCTCCTATCGTTGCCGCAATTATTGTTATAGTTCAGAAAGGAGGTCCTTAC
CTCGCCATCTATCTGTGGGCATTCATGTTTATCCTGTCTCTAGTGATGATGACT
ATATACCCTGTTTTGATTGCACCTCTTTTCAACAAGTTCCTCCTTCTTGAT
GGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCTCTG

```

AAGAAGCTGTTTGTTCGATGGATCTACAAGGTCAAGCCATAGTAATGCTTAC
ATGTATGGTTTCTTCAAGAACAAAAGGATTGTTCTTTATGACACATTGATTGAG
CAGTGCCAGAATGAGAATGAAATTGTGGCGGTTATTGCACACGAGCTGGGACAC
TGGAAGCTGAATCACACTACATACTCGTTCATTGCTGTTCAAATCCTTGCCTTC
TTGCAATTTGGAGGATACACTCTTGTGAGAACTCCACTGATCTCTTCAGGAGT
TTTGGTTTTTGATACACAACCAGTTCTCATTTGGTTTGATCATATTTGAGCACACT
GTAATACCACTTCAACACCTAGTAAGCTTTGACCTCAACCTTGTTAGTCGAGCG
TTTGAGTTTCAGGCTGATGCTTTTGCAGTGAATCTTGGTTATGCAAAGGATCTA
CGTCCTGCCCTAGTGAAGCTACAGGAAGAGAACTTATCAGCGATGAACACAGAC
CCATTGTACTCAGCTTATCACTACTCACACCCTCCTCTTGTAGAGAGGCTTCGA
GCCATTGATGGAGAAGACAAGAAGACAGATTAA

```

A disclosed CPP polypeptide (SEQ ID NO:110) encoded by SEQ ID NO:109 has 424 amino acid residues and is presented in Table 16B using the one-letter amino acid code.

**Table 16B. Encoded CPP protein sequence (SEQ ID NO:110).**

```

MAIPFMETVVGFMIVMYVFETYLDLRQHTALKLPTLPKTLVGVISQEKFEKSRA
YSLDKSHFHFVHEFVTILMDSAILFFGILPFWKISGGFLPMVGLDPENEILHT
LSFLAGLMTWSQITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGILLSVIP
APPIVAAIIVIVQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPD
GDLREKIEKLASSLKFPLKKLFVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQ
QQQNEINEIVAVIAHELGHWKLNHTTYSFIAVQILAFQFGGYTLVRNSTDLFRS
FGFDTQPVLIGLIIFQHTVIPLQHLVSFDLNLVSRAFEFQADAFVNLGYAKDL
RPALVKLQEENLSAMNTDPLYSAHYHSHPPPLVERLRAIDGEDKKT

```

The present invention also includes a nucleic acid sequence complimentary to the *Brassica napus* CaaX prenyl protease of SEQ ID NO:109. The disclosed complimentary sequence is shown as SEQ ID NO:111.

SEQ ID NO:111

```

TTAATCTGTCTTCTTGTCTTCTCCATCAATGGCTCGAAGCCTCTCTACAAGAGGAGGGTGTGAG
TAGTGATAAGCTGAGTACAATGGGTCTGTGTTTCATCGCTGATAAGTTCTCTTCCTGTAGCTTCA
CTAGGGCAGGACGTAGATCCTTTGCATAACCAAGATTCACTGCAAAAGCATCAGCCTGAAACTC
AAACGCTCGACTAACAAGGTTGAGGTCAAAGCTTACTAGGTGTTGAAGTGGTATTACAGTGTGC
TGAAATATGATCAAACCAATGAGAACTGGTTGTGTATCAAAACCAAACTCCTGAAGAGATCAG

```

TGGAGTTTCTGACAAGAGTGTATCCTCCAAATTGCAAGAAGGCAAGGATTTGAACAGCAATGAA  
 CGAGTATGTAGTGTGATTTCAGCTTCCAGTGTCCAGCTCGTGTGCAATAACCGCCACAATTTCA  
 TTCTCATTCTGGCACTGCTGAATCAATGTGTCTATAAAGAACAATCCTTTTGTCTTGAAGAAAC  
 CATACTGTAAGCATTACTATGGCTTGACCTTGTAGATCCATCGACAACAAACAGCTTCTTCAG  
 AGGAACTTTAGAGAAGAAGCAAGTTTCTCAATCTTCTCCCGGAGGTCTCCATCAGGAAGAGGA  
 GTGAAC TTGTTGAAAAGAGGTGCAATCAAAACAGGGTATATAGTCATCATCACTAGAGACAGGA  
 TAAACATGAATGCCCACAGATAGATGGCGAGGTAAGGACCTCCTTTCTGAACTATAACAATAAT  
 TGCGGCAACGATAGGAGGGGCAGGTATGACAGAGAGGAGTATTCCTTTGATCATGTCCCTAATG  
 AACATCCATATTGTTTGTGTTGTTGAACCCATGCCGAGACTCGATCACGAAAGTTGAGTACAAAG  
 AAAATGGCAAATCAGTGATCTGTGACCATGTCTATAAGACCAGCCAAGAATGAAAGAGTGTGCAG  
 GATTTCAATTCTCTGGATCGAGTCCCACCATTTGGTAGAAAGCCGCCAGATATCTTCCAAAACCAA  
 GGCAAGATCCCAAAGAACAGAATCGCAGAGTCCATAAGTATAGTAACAAACTCATGAACAAAGT  
 GAAAATGGCTTTTGTCAAGACTGTAAGCTCGAGATTTCTCAAACCTTCTCTTGGCTAATGACTCC  
 AACCAAAGTCTTTGGGAGAGTGGAAGCTTGAGAGCAGTATGTTGCCTCAGATCCAAATACGTC  
 TCAAAAACGTACATCACTATCATAAAACCAACGACGGTTTCCATGAAAGGAATCGCCAT

Due to the nature of the cloning strategy the sequence presented is not full length but is missing the 5' and 3' non-translated regions. The percent identities of the *Brassica napus* nucleotide sequence and its encoded amino acid sequence to that of other CPP sequences as determined by ClustalW analysis are shown in Figure 26.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

#### ***Glycine max* CPP (GmCPP)**

A disclosed nucleic acid of 1275 nucleotides (SEQ ID NO:112) and also referred to as GmCPP, is shown in Table 17.

**Table 17A. GmCPP Nucleotide Sequence (SEQ ID NO:112).**

ATGGCGTTTCCCTACATGGAAGCCGTTGTTCGGATTTATGATATTAATGTACATT TTTGAAACTTACTTGGATGTGCGACAACATAGGGCCCTCAAACCTCCTACTCTT CCAAAGACTTTAGAGGGTGTATCAGCCAAGAGAAATTTGAGAAATCTAGAGCC TATAGTCTTGATAAAAGCCACTTCCATTTTGTTCACGAGTTTGTGACAATAGTG
--

```

ACAGACTCTACAATTTTGTACTTTGGGGTATTGCCCTGGTTTTTGAAGAAATCA
GGAGATTTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACC
CTTGCCTTCTTAGCAGGGCTGATGATTTGGTCACAGATAACAGATTTGCCCTTT
TCTCTGTACTCAACTTTTGTGATTGAGGCCCGTCATGGTTTTAATAAGCAAACA
CCATGGTTATTCTTTAGGGACATGCTTAAAGGAATTTTCCTTCTGTAATAATT
GGTCCACCTATTGTGGCTGCAATCATTGTAATAGTACAGAAAGGAGGTCCATAC
TTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTCTATTGTGATGATGACC
CTTTATCCAGTACTAATAGCTCCACTCTTCAATAAGTTCACCTCCACTTCAGAT
GGTCAACTCAGGGAGAAAATCGAGAACTTGCTTCCTCCCTCAACTATCCGTTA
AAGAACTATTTGTTGTGCGATGGATCCACAAGATCAAGTCACAGCAATGCCTAT
ATGTATGGATTCTTCAAGAACAAGAGGATTGTCCCTTATGACACATTAATTCAA
CAGTGCAAAGACGATGAGGAAATTGTTGCTGTTATTGCCCATGAGTTGGGACAC
TGGAAGCTCAACCATACTGTGTACACATTTGTTGCTATGCAGATTCTTACACTT
CTACAATTTGGAGGATATACACTAGTGCGAAATTGAGCTGATCTGTATCGAAGC
TTTGGGTTTGATACGCAGCCAGTCCTCATTGGGCTCATCATATTCAGCATACT
GTAATCCCACTTCAGCAATTGGTCAGCTTTGGTCTGAACCTAGTCAGCCGATCA
TTTGAATTTTCAAGGCTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTA
CGCGGTGGTCTTGTGAACTACAGGAGGAGAATCTGTCAGCTATGAATACAGAT
CCTTGGTACTCTGCTTATCACTATTCTCATCCTCCCCTTGTTGAAAGATTGGCC
GCGCTGGACGAACCGGATAAGAAGGAAGACTAA

```

A disclosed CPP polypeptide (SEQ ID NO:113) encoded by SEQ ID NO:112 has 424 amino acid residues and is presented in Table 17B using the one-letter amino acid code.

**Table 17B. Encoded CPP protein sequence (SEQ ID NO:113).**

```

MAFPYMEAVVGFILMYIFETYLDVRQHRALKLPTLPKTLLEGVISQEKFEKSRAYSLDKS
HFHFVHEFVTIVTDSTILYFGVLPWFWKSGDFMTIAGFNAENEILHTLAFLAGLMIWSQ
ITDLPFSLYSTFVIEARHGFNKQTPWLFFRDMLKGIFLSVIIGPPIVAIIIVIVQKGGPY
LAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFPLPDGQLREKIEKLASSLNYPLKKLFV
DGSTRSSHSNAYMYGFFKNKRIVPYDTLIQQCKDDEEIVAVIAHELGHWKLNHTVYTFVA
MQILTLLQFGGYTLVRNSADLYRSFGFDTQPVLIIGLIIFQHTVIPLQLVSFGLNLVSRS
FEFQADGFAKKLGYASGLRGGLVKLQEEENLSAMNTDPWYSAYHYSHPPLVERLAALDEPD
KKED

```



The present invention also includes a nucleic acid sequence complimentary to the *Glycine max* CaaX prenyl protease of SEQ ID NO:112. The disclosed complimentary sequence is shown as SEQ ID NO:114.

## SEQ ID NO:114

TTAGTCTTCCTTCTTATCCGGTTCGTCCAGCGCGGCCAATCTTTCAACAAGGGGAGGATGAGAA  
TAGTGATAAGCAGAGTACCAAGGATCTGTATTCATAGCTGACAGATTCTCCTCCTGTAGTTTCA  
CAAGACCACCGCGTAATCCAGATGCATATCCAAGCTTCTTGGCAAAGCCATCAGCCTGAAATTTC  
AAATGATCGGCTGACTAGGTTTACAGACCAAAGCTGACCAATTGCTGAAGTGGGATTACAGTATGC  
TGAAATATGATGAGCCCAATGAGGACTGGCTGCGTATCAAACCCAAAGCTTCGATACAGATCAG  
CTGAATTTTCGCACTAGTGTATATCCTCCAAATTGTAGAAGTGTAAGAATCTGCATAGCAACAAA  
TGTGTACACAGTATGGTTGAGCTTCCAGTGTCCCAACTCATGGGCAATAACAGCAACAATTTCC  
TCATCGTCTTTGCACTGTTGAATTAATGTGTCATAAGGGACAATCCTCTTGTTCTTGAAGAATC  
CATACATATAGGCATTGCTGTGACTTGATCTTGTGGATCCATCGACAACAAATAGTTTCTTTAA  
CGGATAGTTGAGGGAGGAAGCAAGTTTCTCGATTTTCTCCCTGAGTTGACCATCTGGAAGTGGA  
GTGAACTTATTGAAGAGTGGAGCTATTAGTACTGGATAAAGGGTCATCATCACAATAGAAAGAC  
CAAACGTAAAAACCCAAAGATAGATGGCCAAGTATGGACCTCCTTTCTGTACTATTACAATGAT  
TGCAGCCACAATAGGTGGACCAATTATTACAGAAAGGAAAATTCCTTTAAGCATGTCCCTAAAG  
AATAACCATGGTGTGTTTGCTTATTAAAACCATGACGGGCCTCAATCACAAAAGTTGAGTACAGAG  
AAAAGGGCAAATCTGTTATCTGTGACCAAATCATCAGCCCTGCTAAGAAGGCAAGGGTATGCAG  
TATTTCAATTCTCAGCATTGAAACCAGCTATTGTCATAAAATCTCCTGATTTCTTCCAAAACCAG  
GGCAATACCCCAAAGTACAAAATTGTAGAGTCTGTCACTATTGTCACAAACTCGTGAACAAAAT  
GGAAGTGGCTTTTATCAAGACTATAGGCTCTAGATTTCTCAAATTTCTCTTGGCTGATAACACC  
CTCTAAAGTCTTTGGAAGAGTAGGAAGTTTGAGGGCCCTATGTTGTGCGCACATCCAAGTAAGTT  
TCAAAAATGTACATTAATATCATAAATCCGACAACGGCTTCCATGTAGGGAAACGCCAT

Due to the nature of the cloning strategy the sequence presented is not full length but is missing the 5' and 3' non-translated regions. The percent identities of the *Glycine max* nucleotide sequence and its encoded amino acid sequence to that of other CPP sequences as determined by ClustalW analysis are shown in Figure 26.

Using the sequences disclosed herein as hybridization probes, one is able to screen and isolate full length sequences from cDNA or genomic libraries or use the rapid amplification of cDNA ends (RACE) technology or other such PCR techniques.

The CPP nucleic acids and amino acids disclosed above have homology to other disclosed CPP sequences (GenBank ID NOs: AL161491 (AT4g01320), AF007269 and AF353722; WO 02/16625 A2 ). The homology between these and other sequences is shown in the ClustalW alignment analysis shown in Tables 18A-18B.

Table 18A. ClustalW Nucleic Acid Analysis of CaaX Prenyl Protease

1: PPI-AtCPP	SEQ ID NO:97
2: PPI-BnCPP	SEQ ID NO:109
3: PPI-GmCPP	SEQ ID NO:112
4: BASF_AT1	SEQ ID NO:116
5: BASF_AT2	SEQ ID NO:118
6: BASF-Corn	SEQ ID NO:120
7: BASF-Gm	SEQ ID NO:122
8: AFC1	SEQ ID NO:124
9: AT4g01320	SEQ ID NO:126
10: AF007269	SEQ ID NO:128

CLUSTAL W (1.81) multiple sequence alignment

```

PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269      ATGGCGATTCTTTTCATGGAACCGTCGTGGGTAAGCTTCAAAACCTTTTCTGAGACAT
PPI-AtCPP      -----
BASF_AT2       -----
afc1           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----

```

```

PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269      TTTACTATCCTGTTTCACTCATCGTATTTTCGTTTTTGGTTTGGCTTTCTGTGTTG
PPI-AtCPP      -----
BASF_AT2       -----
afc1           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----

```

```

PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269      TGTGTGTTGAGATTCCATGACTCGTTTGTTCATATACCATCGTCTCTGCTTCTCGTTTC
PPI-AtCPP      -----
BASF_AT2       -----
afc1           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----

```

```

PPI-GmCPP      -----

```

BASF-Gm  
 AT4g01320  
 AF007269 TAAATTTTGTCTTTTCTAATAGTGCGTACCTTGATCTGAGGTTTATTACTCCTACTAG  
 PPI-AtCPP  
 BASF\_AT2  
 afc1  
 BASF\_AT1  
 PPI-BnCPP  
 BASF-Corn

PPI-GmCPP  
 BASF-Gm  
 AT4g01320  
 AF007269 TTTCTTGTCTTACTCGTGCGTTTGATTGATTGAGCTTATGTGATTTCATCATCTCTTC  
 PPI-AtCPP  
 BASF\_AT2  
 afc1  
 BASF\_AT1  
 PPI-BnCPP  
 BASF-Corn

PPI-GmCPP  
 BASF-Gm  
 AT4g01320  
 AF007269 CTCGGTTTTAGAATGTACGGAGCTTCTCTGTAAACCAAAATCTAGGATTGGGAAGAAAA  
 PPI-AtCPP  
 BASF\_AT2  
 afc1  
 BASF\_AT1  
 PPI-BnCPP  
 BASF-Corn

PPI-GmCPP  
 BASF-Gm  
 AT4g01320  
 AF007269 GTCGGAGTCTTTTTTCTCATTCCCGATTGGAAATTGAGAATCTTGAAATTTTCTTT  
 PPI-AtCPP  
 BASF\_AT2  
 afc1  
 BASF\_AT1  
 PPI-BnCPP  
 BASF-Corn

PPI-GmCPP  
 BASF-Gm  
 AT4g01320  
 AF007269 GTTCAAGTCATACAGCTTGAGGTTTGGGTTTCTTGTGAGGGTATTATTATGTTCTGTGA  
 PPI-AtCPP  
 BASF\_AT2  
 afc1  
 BASF\_AT1  
 PPI-BnCPP  
 BASF-Corn

PPI-GmCPP  
 BASF-Gm  
 AT4g01320  
 AF007269 AAGCAGTGGTAACAACGCAGAGTACGCGGGGGAGACGCATGGTTCTGAACTAATTGTTA  
 PPI-AtCPP  
 BASF\_AT2  
 afc1  
 BASF\_AT1  
 PPI-BnCPP

```

BASF-Corn -----

PPI-GmCPP -----
BASF-Gm TAAATAATACCTAAAATTTTGAGTTGTCTTAAACATTGGGGTTTAAACAAATCCAATCTC
AT4g01320 -----
AF007269 AATGTTGCATCAAAACTCTTTTCAGTGCTCCAATGTTTCCATCAGTAGTCAGCACAAAGAGA
PPI-AtCPP -----
BASF_AT2 -----
afc1 -----
BASF_AT1 -----
PPI-BnCPP -----
BASF-Corn -----

PPI-GmCPP -----
BASF-Gm TCAATATAAAACCCAATGATCTCACC--CTCACTCCGTTTCTGATTTCTCACTCTTCGTT
AT4g01320 -----
AF007269 TCTTTTATATCTGGTTGATCAAAAAGTAGATGATGTTATTGAATTTTCAGTGATGGAG
PPI-AtCPP -----
BASF_AT2 -----
afc1 -----
BASF_AT1 -----
PPI-BnCPP -----
BASF-Corn -----

PPI-GmCPP -----ATGGCGTTTCCC--TACATGGAAGCCG
BASF-Gm TCTCGTTCGGTTCATCAGCGTGTGTCTCAGC-CATGGCGTTTCCC--TACATGGAAGCCG
AT4g01320 -----ATGGCGATTCCCT--TTCATGGAACCCG
AF007269 TATCTGTTGTTGTGGCATTAGAGTAGATTTCGTTTTCATCTTCTGTTTATTCTTTTTC
PPI-AtCPP -----ATGGCGATTCCCT--TTCATGGAACCCG
BASF_AT2 -----ATGGCGATTCCCT--TTCATGGAACCCG
afc1 -----ATGGCGATTCCCT--TTCATGGAACCCG
BASF_AT1 -----ATGGCGATTCCCT--TTCATGGAACCCG
PPI-BnCPP -----ATGGCGATTCCCT--TTCATGGAACCCG
BASF-Corn -----

PPI-GmCPP -----
BASF-Gm TTGTCGGATTATGATATTAATGTACATTTTTGAAACTTACTTGGATGTGCGACAACATA
AT4g01320 TTGTCGGATTATGATATTAATGTACATTTTTGAAACTTACTTGGATGTGCGACAACATA
AF007269 TCGTGGGTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
PPI-AtCPP TTACAGGTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
BASF_AT2 TCGTGGGTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
afc1 TCGTGGGTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
BASF_AT1 TCGTGGGTTTATGATAGTGATGTACATTTTTGAGACGTATTTGGATCTGAGGCAACTCA
PPI-BnCPP TCGTTGGTTTATGATAGTGATGTACGTTTTTGGACGTATTTGGATCTGAGGCAACATA
BASF-Corn -----

PPI-GmCPP -----
BASF-Gm GGGCCCTCAAACCTTCCCTACTCTTCCAAAGACTTTAGAGGGTGTATCAGCCAAGAGAAAT
AT4g01320 GGGCCCTCAAACCTTCCCTACTCTTCCAAAGACTTTAGAAGGTGTTATCAGCCAAGAGAAAT
AF007269 CTGCTCTCAAGCTTCCAACCTCTCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
PPI-AtCPP CTGCTCTCAAGCTTCCAACCTCTCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
BASF_AT2 CTGCTCTCAAGCTTCCAACCTCTCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
afc1 CTGCTCTCAAGCTTCCAACCTCTCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
BASF_AT1 CTGCTCTCAAGCTTCCAACCTCTCCGAAAACCTTGGTTGGTGTAATTAGCCAAGAGAAGT
PPI-BnCPP CTGCTCTCAAGCTTCCCACTCTCCCAAGACTTTGGTTGGAGTCATTAGCCAAGAGAAGT
BASF-Corn -----

PPI-GmCPP -----
BASF-Gm TTGAGAAATCTAGAGCCTATAG-----
AT4g01320 TTGAGAAATCTAGAGCCTATAG-----
AF007269 TTGAGAAATCAGGAGCATAACAG-----
PPI-AtCPP TTGAGAAATCAGGAGCATAACAG-----
BASF_AT2 TTGAGAAATCAGGAGCATAACAG-----
afc1 TTGAGAAATCAGGAGCATAACAG-----
BASF_AT1 TTGAGAAATCAGGAGCATAACAG-----

```

```

PPI-BnCPP      TTGAGAAATCTCGAGCTTACAG-----
BASF-Corn      -----

PPI-GmCPP      -----TCTTGATAAA---AGCCA
BASF-Gm        -----TCTTGATAAA---AGCCA
AT4g01320      -----GGATATCATCACTGAGAACTTTAATATATGCAGCTA
AF007269      TTTTAGTTTTTTTATAATTGCCAGGGGATATCATCACTGAGAACTTTAATATATGCAGCTA
PPI-AtCPP      -----TCTTGACAAA---AGCTA
BASF_AT2       -----TCTTGACAAA---AGCTA
afc1           -----TCTTGACAAA---AGCTA
BASF_AT1       -----TCTTGACAAA---AGCTA
PPI-BnCPP      -----TCTTGACAAA---AGCCA
BASF-Corn      -----

PPI-GmCPP      CTTCCATTTTGTTCACGAGTTTGTGACAATAGTGACAGACTCTACAATTTGTACTTTGG
BASF-Gm        CTTCCATTTTGTTCACGAGTTTGTGACAATAGTGACAGACTCTACAATTTGTACTTTGG
AT4g01320      TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
AF007269      TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
PPI-AtCPP      TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
BASF_AT2       TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
afc1           TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
BASF_AT1       TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
PPI-BnCPP      TTTTCACTTTTGTTCATGAGTTTGTAACTATACTTATGGACTCTGCAATTTTGTTCCTTTGG
BASF-Corn      -----

PPI-GmCPP      GGTATTGCCCTGGTTTTTGAAG-----
BASF-Gm        GGTATTGCCCTGGTTTTTGAAG-----
AT4g01320      GATCTTGCCCTGGTTTTTGAAG-----
AF007269      GATCTTGCCCTGGTTTTTGAAGGTACATATCTGGTTTCGGTATACAGTATCTCATTTTGA
PPI-AtCPP      GATCTTGCCCTGGTTTTTGAAG-----
BASF_AT2       GATCTTGCCCTGGTTTTTGAAG-----
afc1           GATCTTGCCCTGGTTTTTGAAG-----
BASF_AT1       GATCTTGCCCTGGTTTTTGAAG-----
PPI-BnCPP      GATCTTGCCCTGGTTTTTGAAG-----
BASF-Corn      -----

PPI-GmCPP      -----AAATCAGGAGAT
BASF-Gm        -----AAATCAGGAGAT
AT4g01320      -----ATGTCTGGAGCT
AF007269      ATATAGAGTTGTTACATTACAATTGTAAAGTTTTCATTTTACCTTAGATGCTGGAGCT
PPI-AtCPP      -----ATGTCTGGAGCT
BASF_AT2       -----ATGTCTGGAGCA
afc1           -----ATGTCTGGAGCT
BASF_AT1       -----ATGTCTGGAGCT
PPI-BnCPP      -----ATATCTGGCGGC
BASF-Corn      -----

PPI-GmCPP      TTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACCCTTGCCTTCTTA
BASF-Gm        TTTATGACAATAGCTGGTTTCAATGCTGAGAATGAAATACTGCATACCCTTGCCTTCTTA
AT4g01320      GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTTATTCTTG
AF007269      GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTTATTCTTG
PPI-AtCPP      GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTTATTCTTG
BASF_AT2       GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTTATTCTTG
afc1           GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTTATTCTTG
BASF_AT1       GTTTTACCGAGGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTTATTCTTG
PPI-BnCPP      TTTCTACCAATGGTGGGACTCGATCCAGAGAATGAAATCCTGCACACTCTTTTATTCTTG
BASF-Corn      -----ACGAGGCTGAGTGTGAGAATGAGATAATACACACCCTTGCTTTCTTA
* * * * *

PPI-GmCPP      GCAGGGCTGATGATTGGTACAG-----
BASF-Gm        GCAGGGCTGATGATTGGTACAG-----
AT4g01320      GCTGGTGTATGACATGGTACAG-----
AF007269      GCTGGTGTATGACATGGTACAGGTTTCCAAATAAACCCCTTCATATAGTCCTATACG
PPI-AtCPP      GCTGGTGTATGACATGGTACAG-----
BASF_AT2       GCTGGTGTATGACATGGTACAG-----
afc1           GCTGGTGTATGACATGGTACAG-----
BASF_AT1       GCTGGTGTATGACATGGTACAG-----
PPI-BnCPP      GCTGGTCTTATGACATGGTACAG-----
BASF-Corn      GCTGGTCCATGGTTGGTCCGAG-----

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PPI-GmCPP -----  
 BASF-Gm -----  
 AT4g01320 -----  
 AF007269 TTTAGCATCAAAATATCTATTTTCTTAAGATAATAATATTTCTTTTATATCTGATGCAG  
 PPI-AtCPP -----  
 BASF\_AT2 -----  
 afc1 -----  
 BASF\_AT1 -----  
 PPI-BnCPP -----  
 BASF-Corn -----

PPI-GmCPP ATAACAGATTGCCCTTTTCTCTGTACTCAACTTTTGTGATTGAGGCCCGTCATGGTTTT  
 BASF-Gm ATAACAGATTGCCCTTTTCTCTGTACTCAACTTTTGTGATTGAGGCCCGTCATGGTTTT  
 AT4g01320 ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 AF007269 ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 PPI-AtCPP ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 BASF\_AT2 ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 afc1 ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 BASF\_AT1 ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 PPI-BnCPP ATCACTGATTGGCATTCTTTTGTACTCAACTTTTCGTGATCGAGTCTCGGCATGGGTTC  
 BASF-Corn ATTACAGACTGCCGTTCTCTCTATTCAACTTTTGTATAGAGGCTCGACATGGTTTT  
 \*\* \*\* \*\* \*\*\*\*\* \*\* \*\* \* \*\* \*\*\*\*\* \*\* \*\* \* \*\* \*\*\*\*\* \*\*

PPI-GmCPP AATAAG-----  
 BASF-Gm AATAAG-----  
 AT4g01320 AACAAA-----  
 AF007269 AACAAAGTATGTCGTATTTCCAACACTACCTTGTGACTTACGTTTTTTTATCAGAGATGT  
 PPI-AtCPP AACAAA-----  
 BASF\_AT2 AACAAA-----  
 afc1 AACAAA-----  
 BASF\_AT1 AACAAA-----  
 PPI-BnCPP AACAAA-----  
 BASF-Corn AACAAG-----  
 \*\* \*\*

PPI-GmCPP -----CAAACACCATGGTTATTCTTTAGGGACA  
 BASF-Gm -----CAAACACCATGGTTATTCTTTAGGGACA  
 AT4g01320 -----CAAACAATATGGATGTTTCATTAGGGACA  
 AF007269 GGATTAAATTTGCTTCTAAATTCTGTTGACAGCAAACAATATGGATGTTTCATTAGGGACA  
 PPI-AtCPP -----CAAACAATATGGATGTTTCATTAGGGACA  
 BASF\_AT2 -----CAAACAATATGGATGTTTCATTAGGGACA  
 afc1 -----CAAACAATATGGATGTTTCATTAGGGACA  
 BASF\_AT1 -----CAAACAATATGGATGTTTCATTAGGGACA  
 PPI-BnCPP -----CAAACAATATGGATGTTTCATTAGGGACA  
 BASF-Corn -----CAAACATATGGCTCTTCATTAGGGATA  
 \*\*\*\*\* \*\* \*\* \* \*\* \*\*\*\*\* \*\*

PPI-GmCPP TGCTTAAAGGAATTTTCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAATCATTG  
 BASF-Gm TGCTTAAAGGAATTTTCTTTTCCGTAATAATTGGTCCACCTATTGTGGCTGCAATCATTG  
 AT4g01320 TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT  
 AF007269 TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT  
 PPI-AtCPP TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT  
 BASF\_AT2 TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT  
 afc1 TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT  
 BASF\_AT1 TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT  
 PPI-BnCPP TGATCAAAGGAATACTCTCTCTGTCTACTAGGCCCTCTATCGTTGCCGCAATTATTG  
 BASF-Corn TGATCAAAGGAATTTTACTATCCATGATATTGGGGCCCAATCGTGGCTGCTATCATCT  
 \*\* \* \*\*\*\*\* \*\* \*\* \* \*\* \*\* \* \*\* \*\* \*\* \*\*

PPI-GmCPP TAATAGTACAG-----  
 BASF-Gm TAATAGTACAG-----  
 AT4g01320 TCATAGTCCAG-----  
 AF007269 TCATAGTCCAGGTTTATGATTCTGGATTCTATTCTGAGTTTTCACATGGATGA  
 PPI-AtCPP TCATAGTCCAG-----  
 BASF\_AT2 TCATAGTCCAG-----  
 afc1 TCATAGTCCAG-----  
 BASF\_AT1 TCATAGTCCAG-----

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PPI-BnCPP      TTATAGTTCAG-----
BASF-Corn      ACATAGTACAG-----
                *****
PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269      CTATTCTCCATTGAGTGTGAGCTTCAAAGTTTTTAGTTTTCTGTGTTAAAAATTTAAATTT
PPI-AtCPP      -----
BASF_AT2       -----
afc1           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----

PPI-GmCPP      -----AAAGGAGGTCCATACTTGCCATC
BASF-Gm        -----AAAGGAGGTCCATACTTGCCATC
AT4g01320      -----AAAGGAGGTCCATTATCTTGCCATC
AF007269      TGCTTCTCTGAGCATGAAGTTTCTATCTTTTCCAGAAAGGAGGTCTTATCTTGCCATC
PPI-AtCPP      -----AAAGGAGGTCTTATCTTGCCATC
BASF_AT2       -----AAAGGAGGTCTTATCTTGCCATC
afc1           -----AAAGGAGGTCTTATCTTGCCATC
BASF_AT1       -----AAAGGAGGTCTTATCTTGCCATC
PPI-BnCPP      -----AAAGGAGGTCTTACCTCGCCATC
BASF-Corn      -----ATTGGAGGACCTTACCTGGCTATA
                * *****
PPI-GmCPP      TATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCTTTATCCAGTACTA
BASF-Gm        TATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCTTTATCCAGTACTA
AT4g01320      TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
AF007269      TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
PPI-AtCPP      TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
BASF_AT2       TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
afc1           TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
BASF_AT1       TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
PPI-BnCPP      TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG
BASF-Corn      TATCTGTGGGTTTTATGTTTGTATTAGCTCTACTGATGATGACAATATACCCATTGTG
                *****
PPI-GmCPP      ATAGCTCCACTCTTCAATAAGTTCACTCCA-----
BASF-Gm        ATAGCTCCACTCTTCAATAAGTTCACTCCA-----
AT4g01320      ATAGCACCGCTCTTCAACAAGTTCACTCCT-----
AF007269      ATAGCACCGCTCTTCAACAAGTTCACTCCTGTGTGTTTCTGTGATGGCCATTTTACAA
PPI-AtCPP      ATAGCACCGCTCTTCAACAAGTTCACTCCT-----
BASF_AT2       ATAGCACCGCTCTTCAACAAGTTCACTCCT-----
afc1           ATAGCACCGCTCTTCAACAAGTTCACTCCT-----
BASF_AT1       ATAGCACCGCTCTTCAACAAGTTCACTCCT-----
PPI-BnCPP      ATGCACTCTTTTCAACAAGTTCACTCCT-----
BASF-Corn      ATAGCTCCTCTGTTCAACAAGTTCACTCCT-----
                ** * * * *
PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269      TTCACTGCTGTTTGCATATGTTGTACCAGACAATATAATCTCCCGCTTTTTTATGGCT
PPI-AtCPP      -----
BASF_AT2       -----
afc1           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----

PPI-GmCPP      ----CTTCCAGATGGTCAACTCAGGGAGAAAATCGAGAACTTGCTTCCTCCCTCAACTA
BASF-Gm        ----CTTCCAGATGGTCAACTCAGGGAGAAAATCGAGAACTTGCTTCCTCCCTCAACTA
AT4g01320      ---CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT
AF007269      ATAGCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT
PPI-AtCPP      ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT
BASF_AT2       ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT
afc1           ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT
BASF_AT1       ----CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT
PPI-BnCPP      ----CTTCTGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT

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BASF-Corn      -----CTTCCTGAAGGAGTCCTCAGGGAAAAAATAGAGAAGCTGGCAGCTTCCCTCAAGTT
                *****
PPI-GmCPP      TCCGTTAAAGAACTATTTGTTGTCGATGGATCCACAAGATCAAGTCACAGCAATG----
BASF-Gm        TCCGTTAAAGAACTATTTGTTGTCGATGGATCCACAAGATCAAGTCACAGCAATG----
AT4g01320      TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
AF007269       TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAG
PPI-AtCPP      TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
BASF_AT2       TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
afc1           TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
BASF_AT1       TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG----
PPI-BnCPP      TCCTCTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGTAATG----
BASF-Corn      TCCTTTGAAAAGCTTTTCGTGGTAGATGGGTCTACCAGATCAAGCCACAGTAATG----
                ***
PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269       AAGCTTGAGATCTCTTCCTACCTACTTTACTCTAGTTTACCATTAGAAGCTTACGTATCT
PPI-AtCPP      -----
BASF_AT2       -----
afc1           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----

PPI-GmCPP      -----CCTATATGTATGGATTCTTCAAGAACAAAGAGGATTGTCCCTTAT
BASF-Gm        -----CCTATATGTATGGATTCTTCAAGAACAAAGAGGATTGTCCCTTAT
AT4g01320      -----CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
AF007269       TGTACATCATACAGGCTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
PPI-AtCPP      -----CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
BASF_AT2       -----CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
afc1           -----CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
BASF_AT1       -----CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT
PPI-BnCPP      -----CTTACATGTATGGTTTCTTCAAGAACAAAAGGATTGTTCTTTAT
BASF-Corn      -----CCTACATGTATGGTTTCTTCAAGAACAAAGCGCATAGTACTCTAT
                *
PPI-GmCPP      GACACATTAATTCAACAG-----
BASF-Gm        GACACATTAATTCAACAG-----
AT4g01320      GATACGTTGATTTCAGCAG-----
AF007269       GATACGTTGATTTCAGCAGGTTACTGTGACTCTTGATGCTTCAAACGAGCTATACTCACATT
PPI-AtCPP      GATACGTTGATTTCAGCAG-----
BASF_AT2       GATACGTTGATTTCAGCAG-----
afc1           GATACGTTGATTTCAGCAG-----
BASF_AT1       GATACGTTGATTTCAGCAG-----
PPI-BnCPP      GACACATTGATTTCAGCAG-----
BASF-Corn      GACACATTGATTTCAGCAG-----
                **
PPI-GmCPP      -----TGCAAAGACGATGAGG
BASF-Gm        -----TGCAAAGACGATGAGG
AT4g01320      -----TGCAAGAATGAGGATG
AF007269       TCTGTTTCTGGTTCTGAAACATAACATAATCTTCTATTGTGTCAGTGCAAGAATGAGGATG
PPI-AtCPP      -----TGCAAGAATGAGGATG
BASF_AT2       -----TGCAAGAATGAGGATG
afc1           -----TGCAAGAATGAGGATG
BASF_AT1       -----TGCAAGAATGAGGATG
PPI-BnCPP      -----TGCCAGAATGAGAATG
BASF-Corn      -----TGTAGCAATGAGGATG
                **
PPI-GmCPP      AAATTGTTGCTGTTATTGCCCATGAGTTGGGACACTGGAAGCTCAACCATACTGTGTACA
BASF-Gm        AAATTGTTGCTGTTATTGCCCATGAGTTGGGACACTGGAAGCTCAACCATACTGTGTACA
AT4g01320      AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT
AF007269       AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT
PPI-AtCPP      AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT
BASF_AT2       AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT
afc1           AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT
BASF_AT1       AAATTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT

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PPI-BnCPP      AAATTGTGGCGTTATTGCACACGAGCTGGGACACTGGAAGCTGAATCACACTACATACT
BASF-Corn      AGATAGTTTCTGTTATAGCACATGAACTTGGACACTGGAAACTCAATCATACTGTCTATT
* * * * *
PPI-GmCPP      CATTGTGTGCTATGCAG-----
BASF-Gm        CATTGTGTGCTATGCAG-----
AT4g01320      CGTTCATTGCAGTTCAA-----
AF007269       CGTTCATTGCAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACCTACTCACATCTACAT
PPI-AtCPP      CGTTCATTGCAGTTCAA-----
BASF_AT2       CGTTCATTGCAGTTCAA-----
afc1          CGTTCATTGCAGTTCAA-----
BASF_AT1       CGTTCATTGCAGTTCAA-----
PPI-BnCPP      CGTTCATTGTCTGTTCAA-----
BASF-Corn      CCTTGTAGCTGTCCAG-----
* * * * *

PPI-GmCPP      -----ATTCTTACA
BASF-Gm        -----ATTCTTACA
AT4g01320      -----ATCCTTGCC
AF007269       TTCACTTAAGAAATCATGTCTTATGACCCTCTCTCAATGTTTGTCTGCAGATCCTTGCC
PPI-AtCPP      -----ATCCTTGCC
BASF_AT2       -----ATCCTTGCC
afc1          -----ATCCTTGCC
BASF_AT1       -----ATCCTTGCC
PPI-BnCPP      -----ATCCTTGCC
BASF-Corn      -----CTGCTTATG
* * * * *

PPI-GmCPP      CTTCTACAATTGGAGGATATACACTAGTGCAGAAATTCAGCTGATCTGTATCGAAGCTTT
BASF-Gm        TTCTTACAATTGGAGGATATACACTAGTGCAGAAATTCAGCTGATCTGTATCGAAGCTTT
AT4g01320      TTCTTACAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
AF007269       TTCTTACAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
PPI-AtCPP      TTCTTACAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
BASF_AT2       TTCTTACAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
afc1          TTCTTACAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
BASF_AT1       TTCTTACAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
PPI-BnCPP      TTCTTGCAATTGGAGGATACACTCTGTGCAGAACTCCACTGATCTCTTCAGGAGTTTC
BASF-Corn      TTTCTTCAATTGGAGGATATACTCTAGTAAGGAGCTCAAAGATCTATTTGGAAGTTTT
* * * * *

PPI-GmCPP      GGGTTTGATACGCAGCCAGTCCTCATTTGGGCTCATCATATTTTCAG-----
BASF-Gm        GGGTTTGATACGCAGCCAGTCCTCATTTGGGCTCATCATATTTTCAG-----
AT4g01320      GGATTTGATACACAGCCTGTTCTCATTTGGTTTGATCATATTTTCAG-----
AF007269       GGATTTGATACACAGCCTGTTCTCATTTGGTTTGATCATATTTTCAGAGTTTGTATTTTTCG
PPI-AtCPP      GGATTTGATACACAGCCTGTTCTCATTTGGTTTGATCATATTTTCAG-----
BASF_AT2       GGATTTGATACACAGCCTGTTCTCATTTGGTTTGATCATATTTTCAG-----
afc1          GGATTTGATACACAGCCTGTTCTCATTTGGTTTGATCATATTTTCAG-----
BASF_AT1       GGATTTGATACACAGCCTGTTCTCATTTGGTTTGATCATATTTTCAG-----
PPI-BnCPP      GGTTTGGATACACAACCAGTTCTCATTTGGTTTGATCATATTTTCAG-----
BASF-Corn      GGCTTCAAGGACCAGCCAGTAATAATTGGATTGATCATTTTCCCG-----
* * * * *

PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269       CTTTGTACACTAATCTAATGAATCAAGGATGGATTAAAGAAAAAACTCTAAACCTTTG
PPI-AtCPP      -----
BASF_AT2       -----
afc1          -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----
PPI-GmCPP      -----CATACTGTAATCCCACTTCAGCAATTGGTCAGC
BASF-Gm        -----CATACTGTAATCCCACTTCAGCAATTGGTCAGC
AT4g01320      -----CACACTGTAATACCACTGCAACATCTAGTAAGC
AF007269       GTTATATCTCTGTCTGATTATCAGCACACTGTAATACCACTGCAACATCTAGTAAGC
PPI-AtCPP      -----CACACTGTAATACCACTGCAACATCTAGTAAGC
BASF_AT2       -----CACACTGTAATACCACTGCAACATCTAGTAAGC
afc1          -----CACACTGTAATACCACTGCAACATCTAGTAAGC
BASF_AT1       -----CACACTGTAATACCACTGCAACATCCAGTAAGC

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PPI-BnCPP      -----CACACTGTAATACCACTTCAACACCTAGTAAGC
BASF-Corn      -----CACACCATAAATACCCATCCAACACCTTCTGAGC
                  ** ** * ** * * ** * **
PPI-GmCPP      TTTGGTCTGAACCTAGTCAGCCGATCATTTGAATTTTCAGG-----
BASF-Gm        TTTGGTCTGAACCTAGTCAGCCGATCATTTGAATTTTCAGG-----
AT4g01320      TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG-----
AF007269       TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG-----
PPI-AtCPP      TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG-----
BASF_AT2       TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG-----
afcl           TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG-----
BASF_AT1       TTTGGCCTCAACCTTGTTAGTCGAGCGTTTGAGTTTCAGG-----
PPI-BnCPP      TTTGACCTCAACCTTGTTAGTCGAGCGTTTGAGTTTCAGG-----
BASF-Corn      TTTGCGCTGAACCTTGTCAGCAGAGCATTTGAATTTTCAGG-----
                  *** ** * ** * ** * ** * ** * **
PPI-GmCPP      -----
BASF-Gm        -----
AT4g01320      -----
AF007269       AGATCCAACCATAGTTTCTTTATTGCAATGGCAGCCTCATCTACTAATCTGAGTTAACGT
PPI-AtCPP      -----
BASF_AT2       -----
afcl           -----
BASF_AT1       -----
PPI-BnCPP      -----
BASF-Corn      -----
PPI-GmCPP      -----CTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTACGCGGTG
BASF-Gm        -----CTGATGGCTTTGCCAAGAAGCTTGGATATGCATCTGGATTACGCGGTG
AT4g01320      -----CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
AF007269       TCCTTTTGCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
PPI-AtCPP      -----CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
BASF_AT2       -----CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
afcl           -----CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG
BASF_AT1       -----CTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTA
PPI-BnCPP      -----CTGATGCTTTTGCAGTGAATCTTGGTTATGCAAAAGATCTACGTCCTG
BASF-Corn      -----CTGATGCTTTTGCCAAGAACCTTGGATATGCCCTCAGCTCCGAGCAG
                  ***** ** * ** * ** * ** * **
PPI-GmCPP      GTCTTGTGAAACTACAGG-----
BASF-Gm        GTCTTGTGAAACTACAGG-----
AT4g01320      CTCTAGTGAAACTACAGGTGAGAGATAAACAACAGAACACAACTGTTACCTCAATTT
AF007269       CTCTAGTGAAACTACAGGTGAGAGATAAACAACAGAACACAACTGTTACCTCAATTT
PPI-AtCPP      CTCTAGTGAAACTACAGG-----
BASF_AT2       CTCTAGTGAAACTACAGG-----
afcl           CTCTAGTGAAACTACAGG-----
BASF_AT1       CTCTAGTGAAACTACAGG-----
PPI-BnCPP      CCCTAGTGAAAGCTACAGG-----
BASF-Corn      CCCTTGTGTTAAACTACAGG-----
                  ** ** * ** * ** * **
PPI-GmCPP      -----AGGAGAATCTGTGAGCTA
BASF-Gm        -----AGGAGAATCTGTGAGCTA
AT4g01320      GTGTACACACTTAAATGGATTTTTTGTGGGATTTTGAGGAAGAGAACTTATCAGCAA
AF007269       GTGTACACACTTAAATGGATTTTTTGTGGGATTTTGAGGAAGAGAACTTATCAGCAA
PPI-AtCPP      -----AAGAGAACTTATCAACAA
BASF_AT2       -----AAGAGAACTTATCAGCAA
afcl           -----AAGAGAACTTATCAGCAA
BASF_AT1       -----AAGAGAACTTATCAGCAA
PPI-BnCPP      -----AAGAGAACTTATCAGCGA
BASF-Corn      -----AGGAGAACTTGTCTGCGA
                  * ** * ** * **
PPI-GmCPP      TGAATACAGATCCTTGGTACTCTGCTTATCACTATTCTCATCCTCCCCTTGTGAAAGAT
BASF-Gm        TGAATACAGATCCTTGCT--CGTGCCG-----
AT4g01320      TGAACACTGATCCATTGTAAGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC
AF007269       TGAACACTGATCCATTGTAAGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC
PPI-AtCPP      TGAACACTGATCCATTGTAAGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC
BASF_AT2       TGAACACTGATCTATTGTAAGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC
afcl           TGAACACTGATCCATTGTAAGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC

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BASF_AT1      TGAATACTGATCCATTGTACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC
PPI-BnCPP     TGAACACAGACCCATTGTACTCAGCTTATCACTACTCACACCTCCTCTTGTAGAGAGGC
BASF-Corn     TGAACACCGATCCTTGGTATTGGCATATCACTACTCCCACCCACCCTCGTCGAGAGGC
              ****  **  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

PPI-GmCPP     TGGCCGCGCTGGACGA---ACCGGATAAGAAGGAAGACTAA-----
BASF-Gm       -----
AT4g01320     TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA-----
AF007269      TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA-----
PPI-AtCPP     TTCGAGCCACTGATGG---AGAAGACAAGAAGACAGATTAA-----
BASF_AT2      TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA-----
afc1          TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA-----
BASF_AT1      TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA-----
PPI-BnCPP     TTCGAGCCATTGATGG---AGAAGACAAGAAGACAGATTAA-----
BASF-Corn     TGCAAGCTTTGGAAGATTGAGACGACAAAAAAGAAGATTAGTCGATCCTTGTATGAGGTT

PPI-GmCPP     -----
BASF-Gm       -----
AT4g01320     -----
AF007269      -----
PPI-AtCPP     -----
BASF_AT2      -----
afc1          -----
BASF_AT1      -----
PPI-BnCPP     -----
BASF-Corn     TACATATGGATTTTCCCTGCCACATGCACACCGATTGAGTGCTTGGATGGTGAGGTTT

PPI-GmCPP     -----
BASF-Gm       -----
AT4g01320     -----
AF007269      -----
PPI-AtCPP     -----
BASF_AT2      -----
afc1          -----
BASF_AT1      -----
PPI-BnCPP     -----
BASF-Corn     TGACATAGGAGTGTTGTCAAAGCTTTAGAGTGCATCTTTCGGTCAGGTGCAACAGCCTTT

PPI-GmCPP     -----
BASF-Gm       -----
AT4g01320     -----
AF007269      -----
PPI-AtCPP     -----
BASF_AT2      -----
afc1          -----
BASF_AT1      -----
PPI-BnCPP     -----
BASF-Corn     CCGTCATTGAGACATATAAGCGAATTAGCTATTAAAAAAAACAGAACTGTTGCATCAAAA

PPI-GmCPP     -----
BASF-Gm       -----
AT4g01320     -----
AF007269      -----
PPI-AtCPP     -----
BASF_AT2      -----
afc1          -----
BASF_AT1      -----
PPI-BnCPP     -----
BASF-Corn     AAAAAAAAAAAAAAGAAACAAAAAAAAAAAAAAAAAAAAAAAAAAGAAAAAAAAAAAAAAAAA

PPI-GmCPP     -----
BASF-Gm       -----
AT4g01320     -----
AF007269      -----
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PPI-AtCPP      -----
BASF_AT2      -----
afcl          -----
BASF_AT1      -----
PPI-BnCPP     -----
BASF-Corn     AAAAAAGTGCTCTGCGTTGTTACCACTGCTTGCCCTATAGTGATCGTATCAGA

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Table 18B. ClustalW Amino Acid Analysis of CaaX Prenyl Protease

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1: PPI-AtCPP      SEQ ID NO:98
2: PPI-BnCPP      SEQ ID NO:110
3: PPI-GmCPP      SEQ ID NO:113
4: BASF_AT1       SEQ ID NO:117
5: BASF_AT2       SEQ ID NO:119
6: BASF-Corn      SEQ ID NO:121
7: BASF-Gm        SEQ ID NO:123
8: AFC1           SEQ ID NO:125
9: AT4g01320      SEQ ID NO:127
10: AF007269      SEQ ID NO:129

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PPI-GmCPP      MAFPYMEAVVGFMIIMYIFETYLDVRQHRALKLPTLPKTLEG-----VISQEKFEKSR
BASF-Gm        MAFPYMEAVVGFMIIMYIFETYLDVRQHRALKLPTLPKTLEG-----VISQEKFEKSR
AF007269      MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLI-----VISQEKFEKSR
AT4g-AtCPP     MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVGVISQEKFEKSRAYRDIIT
BASF_AT2       MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVG-----VISQEKFEKSR
AFC1          MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVG-----VISQEKFEKSR
BASF_AT1       MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVG-----VISQEKFEKSR
PPI-AtCPP     MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVG-----VISQEKFEKSR
PPI-BnCPP     MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVG-----VISQEKFEKSR
BASF-Corn     MAIPFMETVVGFMIIMYIFETYLDLRQLTALKLPTLPKTLVG-----VISQEKFEKSR

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PPI-GmCPP      AYSLDKSHFHFVHEFVTIVTDSTILYFGVLPWFWKSGDFMTIAGFNAENEILHTLAFLA
BASF-Gm        AYSLDKSHFHFVHEFVTIVTDSTILYFGVLPWFWKSGDFMTIAGFNAENEILHTLAFLA
AF007269      -----
AT4g-AtCPP     ENFNICSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
BASF_AT2       AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
AFC1          AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
BASF_AT1       AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
PPI-AtCPP     AYSLDKSYFHFVHEFVTILMDSAILFFGILPWFWKMSGAVLPRLGLDPENEILHTLSFLA
PPI-BnCPP     AYSLDKSHFHFVHEFVTILMDSAILFFGILPWFWKISGGFLPMVGLDPENEILHTLSFLA
BASF-Corn     -----TRLSAENEIHTLAFLA

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PPI-GmCPP      GLMIWSQITDLPFSLYSTFVIEARHGfNKQTPWLFFRDMLKGIFLSVIGPPIVAAIIVI
BASF-Gm        GLMIWSQITDLPFSLYSTFVIEARHGfNKQTPWLFFRDMLKGIFLSVIGPPIVAAIIVI
AF007269      -----TDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
AT4g-AtCPP     GVMTWSQITDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
BASF_AT2       GVMTWSQITDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
AFC1          GVMTWSQITDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
BASF_AT1       GVMTWSQITDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
PPI-AtCPP     GVMTWSQITDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIFI
PPI-BnCPP     GLMTWSQITDLPFSLYSTFVIESRHGfNKQTIWMFIRDMIKGILLSVIPAPPPIVAAIIVI
BASF-Corn     GSMVWSQITDLPFSLYSTFVIESRHGfNKQTIWLFIRDMIKGILLSMILGPPIVAAIYI

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PPI-GmCPP      VQKGGPYLAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGDLREKIEKLASSLNYP
BASF-Gm        VQKGGPYLAIYLWVFTFGLSIVMMTLYPVLIAPLFNKFTPLPDGDLREKIEKLASSLNYP
AF007269      VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
AT4g-AtCPP     VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP
BASF_AT2       VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFP

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*Arabidopsis thaliana*. Wild type plants were grown under standard conditions until the plant has both developing flowers and open flowers. The plant was inverted for 2 minutes into a solution of *Agrobacterium* culture carrying the appropriate gene construct. Plants were then left horizontal in a tray and kept covered for two days to maintain humidity and then righted and bagged to continue growth and seed development. Mature seed was bulk harvested.

Transformed T1 plants were selected by germination and growth on MS plates containing 50 µg/ml kanamycin. Green, kanamycin resistant (Kan<sup>R</sup>) seedlings were identified after 2 weeks growth and transplanted to soil. Plants were bagged to ensure self fertilization and the T2 seed of each plant harvested separately. During growth of T1 plants leaf samples were harvested, DNA extracted and Southern blot and PCR analysis performed.

T2 seeds were analysed for Kan<sup>R</sup> segregation. From those lines that showed a 3:1 resistant phenotype, surviving T2 plants were grown, bagged during seed set, and T3 seed harvested from each line. T3 seed was again used for Kan<sup>R</sup> segregation analysis and those lines showing 100% Kan<sup>R</sup> phenotype were selected as homozygous lines. Further molecular and physiological analysis was done using T3 seedlings.

Transgenic *Brassica napus*, *Glycine max* and *Zea maize* plants were produced using *Agrobacterium* mediated transformation of cotyledon petiole tissue. Seeds were sterilized as follows. Seeds were wetted with 95% ethanol for a short period of time such as 15 seconds. Approximately 30 ml of sterilizing solution I was added (70% Javex , 100µl Tween20) and left for approximately 15 minutes. Solution I was removed and replaced with 30 ml of solution II (0.25% mercuric chloride, 100µl Tween20) and incubated for about 10 minutes. Seeds were rinsed with at least 500 ml double distilled sterile water and stored in a sterile dish. Seeds were germinated on plates of 1/2 MS medium, pH 5.8, supplemented with 1% sucrose and 0.7% agar. Fully expanded cotyledons were harvested and placed on Medium I (Murashige minimal organics (MMO), 3% sucrose, 4.5 mg/L benzyl adenine (BA), 0.7% phytoagar, pH5.8). An *Agrobacterium* culture containing the nucleic acid construct of interest was grown for 2 days in AB Minimal media. The cotyledon explants were dipped such that only the cut portion of the petiole is contacted by the *Agrobacterium* solution. The explants were then embedded in Medium I and maintained for 5 days at 24°C, with 16,8 hr light dark cycles.

Explants were transferred to Medium II (Medium I, 300 mg/L timentin,) for a further 7 days and then to Medium III (Medium II, 20 mg/L kanamycin). Any root or shoot tissue which had developed at this time was dissected away. Transfer explants to fresh plates of Medium III

after 14 -21 days. When regenerated shoot tissue developed the regenerated tissue was transferred to Medium IV (MMO, 3% sucrose, 1.0% phytoagar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin). Once healthy shoot tissue developed shoot tissue dissected from any callus tissue was dipped in 10X IBA and transferred to Medium V (Murashige and Skooge (MS), 3% sucrose, 0.2 mg/L indole butyric acid (IBA), 0.7% agar, 300 mg/L timentin, 20 mg/L 20 mg/L kanamycin) for rooting. Healthy plantlets were transferred to soil. The above method, with or without modifications, is suitable for the transformation of numerous plant species including *Glycine max*, *Zea maize* and cotton.

Transgenic *Glycine max*, *Zea maize* and cotton can be produced using *Agrobacterium*-based methods which are known to one of skill in the art. Alternatively one can use a particle or non-particle biolistic bombardment transformation method. An example of non-particle biolistic transformation is given in U.S. Patent Application 20010026941. This method has been used to produce transgenic *Glycine max* and *Zea maize* plants. Viable plants are propagated and homozygous lines are generated. Plants are tested for the presence of drought tolerance, physiological and biochemical phenotypes as described elsewhere.

The following table identifies the constructs and the species which they have been transformed.

**Table 19** Transformation List

<u>SEQ ID NO:</u>	<u>Construct</u>	<u>Species Transformed</u>
99	pBII121-AtCPP	<i>A. thaliana</i> , <i>B. napus</i>
100	pBII121-HP-AtCPP	<i>A. thaliana</i>
131	pRD29A-AtCPP	<i>A. thaliana</i> , <i>B. napus</i>
132	pRD29A-HP-AtCPP	<i>A. thaliana</i>
134	MuA-AtCPP	<i>Glycine max</i> , <i>Zea mays</i>

Non-limiting examples of vector constructs suitable for plant transformation are given in SEQ ID NO: 99, 5, 35-53.

SEQ ID NO:99

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SEQ ID NO:99 is the nucleic acid sequence of pBI121-AtCPP. *Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter and bolded sequence is the AtCPP sense sequence.*

SEQ ID NO:100

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 cggaaccaccatcaaacaggatttccgctgctggggcaaacacagcgtggaccgcttgctgcaa  
 ctctctcagggccaggcgggtgaagggaatcagctgttgcccgctctcactggtgaaaagaaaa  
 ccacccagctacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgttta  
 caccacaataatactctgcca

SEQ ID NO:100 is the nucleic acid sequence of pBI121-HP-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter and bolded sequence is the AtCPP anti-sense sequence. Sequence in upper case is the truncated GUS fragment. Sequence in bold and underlined is the AtCPP sense sequence.

SEQ ID NO:130

gtttacccgccaatatatctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc  
 tgatcatgagcggagaattaagggagtcacgttatgacccccgccgatgacgcgggacaagccg  
 ttttacgtttggaactgacagaaccgcaacgttgaaggagccactcagccgcgggttctggag  
 tttaatgagctaagcacatacgtcagaaaccattattgcgcgttcaaaagtcgcctaagggtcac  
 tatcagctagcaaatatttcttgtcaaaaatgctccactgacgttccataaattccccctcggt  
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atgactgggcacaaacagacaatcggctgctctgatgccgccgtgttcggctgtcagcgcaggg  
gcgcccgggttctttttgtcaagaccgacctgtccgggtgccctgaatgaactgcaggacgaggca  
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tctaaaaaggtagttcccactgaatcaaaggccatggagtc aaagattcaa atagaggacctaa  
cagaactcgccgtaaagactggcgaaacagttcatacagagtctcttacgactcaatgacaagaa  
gaaaatcttcgtcaacatgggtggagcacgacacacttgctactccaaaaatatcaaagataca  
gtctcagaagaccaaaagggcaattgagacttttcaacaaagggtaatatccggaaacctcctcg  
gattccattgcccagctatctgtcactttattgtgaagatagtggaaaaggaaggtggctccta  
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aaagatggacccccaccacgaggagcatcggtgaaaaagaagacggtccaaccacgtcttcaa  
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TGTGCTGAAATATGATCAAACCAATGAGAACAGGCTGTGTATCAAATCCGAAACTCCTGAAGAG  
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ATGAACGAGTATGTAGTGTGATT CAGTTTCCAATGTCCAAGCTCGTGTGCAATAACCGCCACAA  
TTTCATCCTCATTCTTGCACTGCTGAATCAACGTATCATAAAGAACAATCCTTTTGTTCTTAAA  
GAAACCATACATGTAAGCATTGCTATGGCTTGACCTTGTAGATCCATCGACAACAAACAGCTTC  
TTCAAAGGAAACTTTAGGGAAGAAGCAAGTTTCTCAATCTTCTCCCGAGGTCTCCATCTGGAA  
GAGGAGTGAATTTGTTGAAGAGCGGTGCTATCAAGACCGGGTATATAGTCATCATCACTAGAGA  
CAGGATAAACATGAATGCCCCACAGATAGATGGCAAGATAAGGACCTCCTTTCTGGACTATGAAA  
ATTATCGCAGCAACAATGGGTGGGCCTAGTATGACAGAGAGGAATGTTTCTTTGATCATGTCCC  
TAATGAACATCCATATTGTTTGTGTTGTTGAACCCATGCCGAGACTCGATCACGAAAGTTGAGTA  
CAAAGAAAATGGCAAATCAGTGATCTGTGACCATGTCATAACACCAGCCAAGAATGAAAGAGTA  
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ACCAAGGCAAGATCCCAAAGAACAAAATTGCAGAGTCCATAAGTATAGTTACAAACTCATGAAC  
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atacgcgcatagaaaacaaaatatagcgcgcgcaaactaggataaattatcgcgcgcggtgtcatct  
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 ctactggtgaaaagaaaaaccacccagtagacattaaaaacgtccgcaatgtgttattaagttg  
 tctaagcgtcaatttggtttacaccacaatatatcctgccca

SEQ ID NO:130 is the nucleic acid sequence of pBI121-antisense-AtCPP. *Italicized*  
 sequences are the right and left border repeats. Underlined sequence is the 35S promoter.  
 Sequence in upper case is the AtCPP anti-sense sequence.

SEQ ID NO:131

gtttaccgcgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc  
 tgatcatgagcggagaattaaggagtcacgttatgacccccgccgatgacgcgggacaagccg  
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 ggaccgcttgctgcaactctctcagggccaggcggatgaagggcaatcagctgttgcccgctctca  
 ctggtgaaaagaaaaaccaccccagtacattaaaaacgtccgcaatgtgttattaagttgtcta  
 agcgtcaatttggtttacaccacaatatatcctgccca

SEQ ID NO:131 is the nucleic acid sequence of RD29A-AtCPP. *Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the AtCPP sense sequence.*

SEQ ID NO:132

*gtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc*  
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*ttttacgtttggaactgacagaaccgcaacggtgaaggagccactcagccgcgggtttctggag*  
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 ccagtagattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttggtttacacc  
 acaatatatcctgcc

SEQ ID NO:132 is the nucleic acid sequence of RD29A-HP-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the AtCPP anti-sense sequence. Upper case sequence represents the truncated GUS fragment. Bold and underlined sequence represents the *A. thaliana* CaaX prenyl protease sense fragment.

#### SEQ ID NO:133

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SEQ ID NO:133 is the nucleic acid sequence of RD29A-antisense-AtCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in upper case sequence is the AtCPP anti-sense sequence.

SEQ ID NO:134

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 gaaccaccatcaaacaggattttcgctgctggggcaaaccagcgtggaccgcttgctgcaact  
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 accccagtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttggtttaca  
 ccacaatatatcctgccca

SEQ ID NO:134 is the nucleic acid sequence of MuA-AtCPP. Italicized sequences are the right and left border repeats. Sequence in upper case is the MuA promoter. The *A. thaliana* CaaX prenyl protease sense sequence is in bold.

SEQ ID NO:135

gtttaccgcgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc  
 tgatcatgagcgggagaattaaggaggtcacgttatgacccccgccgatgacgcgggacaagccg

ttttacgtttggaaactgacagaaccgcaacggtgaaggagccactcagccgcgggtttctggag  
ttaaataagcctaagcacatacgtcagaaaccattattgcgcggtcaaaagtcgcctaagggtcac  
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gcgcccgggttcttttgtcaagaccgacctgtccggtgccctgaatgaactgcaggacgaggca  
gcgcggctatcgtggctggccacgacgggcgttccctgcgagctgtgctcgacgttgtcactg  
aagcgggaagggaactggctgctattgggcgaagtgccggggcaggatctcctgtcatctcacct  
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*caccacaatatatcctgcca*

SEQ ID NO:135 is the nucleic acid sequence of MuA-GmCPP. Italicized sequences are the right and left border repeats. Sequence in upper case is the MuA promoter. The *G. max* CaaX prenyl protease sense sequence is in upper case and bold.

#### SEQ ID NO:136

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cccgtctcactgggtgaaaagaaaaaccaccccagttacattaaaaacgtccgcaatgtgttatta  
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SEQ ID NO:135 is the nucleic acid sequence of pBI121-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. The *G. max* CaaX prenyl protease sense sequence is in bold.

SEQ ID NO:137

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 ccagtagacattaaaaacgtccgcaatgtgttattaagtgtctaagcgtcaatttggttacacc  
 acaatatatcctgcca

SEQ ID NO:137 is the nucleic acid sequence of pBI121-HP-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Bold sequence is the antisense prenyl protease fragment of *G. max*. Bold and underlined sequence is the *G. max* sense prenyl protease fragment and sequence in upper case is the truncated GUS fragment.

SEQ ID NO:138

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SEQ ID NO:138 is the nucleic acid sequence of pBI121-antisense-GmCPP. *Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold is the GmCPP anti-sense sequence.*

## SEQ ID NO:139

gtttaccgcgccaatataatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc  
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tctcactggtgaagaaaaaaccacccagctacattaaaaacgtccgcaatgtgttattaagtt  
gtctaagcgtcaatttgtttacaccacaatatatcctgcca

SEQ ID NO:139 is the nucleic acid sequence of pRD29A-GmCPP. *Italicized sequences* are the right and left border repeats. *Underlined sequence* is the RD29A promoter. Sequence in **bold** is the GmCPP sense sequence.

SEQ ID NO:140

gtttacccgccaatatatcctgtcaaactgatagtttaaactgaaggcgggaaacgacaatc  
tgatcatgagcgggagaattaagggagtcacgttatgacccccgccgatgacgcgggacaagccg  
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**acttgatcttgtggatccccATCTACCCGCTTCGCGTCGGCATCCGGTCAGTGGCAGTGAAGGG**  
**CGAACAGTTCCTGATTAACCACAAACCGTTCTACTTTACTGGCTTTGGTCGTCATGAAGATGCG**  
**GACTTGCGTGGCAAAGGATTCGATAACGTGCTGATGGTGCACGACCACGCATTAATGGACTGGA**  
**TTGGGGCCAACTCCTACCGTACCTCGCATTACCTTACGCTGAAGAGATGCTCGACTGGGCAGA**

TGAACATGGCATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTTCGCTCTCTTTAGGCATT  
GGTTTCGAAGCGGGCAACAAGCCGAAAGAACTGTACAGCGAAGAGGCAGTCAACGGGGAAACTC  
AGCAAGCGCACTTACAGGCGATTAAAGAGCTGATAGCGCGTGACAAAAACCACCCAAGCGTGGT  
GATGTGGAGTATTGCCAACGAACCGGATACCCGTCCGCAAGGTGCACGGGAATATTTTCGCGCCA  
CTGGCGGAAGCAACGCGTAAACTCGACCCGACGCGTCCGATCACCTGCGTCAATGTAATGTTCT  
GCGACGCTCACACCGATACCATCAGCGATCTCTTTGATGTGCTGTGCCTGAACCGTTATTACGG  
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TGCGACCTCGCAAGGCATATTGCGCGTTGGCGGTAACAAGAAAGGGATCTTCACTCGCGACCGC  
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ccatcaaacaggattttcgccctgctggggcaaaccagcgtggaccgcttgctgcaactctctca  
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gtacattaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaatttgtttacaccacaa  
tataatcctgcca

SEQ ID NO:140 is the nucleic acid sequence of pRD29A-HP-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the GmCPP antisense sequence, bold and underlined sequence is the GmCPP sense sequence.

SEQ ID NO:141

*gtttacccgccaatatatactgtcaaacactgatagtttaaaactgaaggcgggaaacgacaatctgatcatgagcggagaattaagg  
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taaataatcataccgacatcagtttgaagaaaagggaataaataaataaagatatactaccgacatgagttcca*

**aaaagcaaaaaaaaaagatcaagccgacacagacacgcgtagagagcaaaatgactttgacgtcacaccagaaaacagacgc**  
**ttcatagctgtccctttatctctctcagttctctataaaacttagtgagaccctcctctgttttactcacaatatgcaaaactagaaaaac**  
**aatcatcaggaataaagggtttgattacttctattggaaaggactctagaggatccccgggttagtcttcctttatccggttcgtccag**  
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 gttcacaagaccaccgcgtaatccagatgcataccaagcttctggcagaagccatcagcctgaaattcaaatgatcggctgactaggtca  
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 ccgcttgcgtgcaactctctcagggccaggcggtgaagggaatcagctgttgcctgtcactgggtgaaagaaaaaccaccca  
 gtacattaaaaacgtccgcaatgtgttattaaagtgtctaagcgtcaatttgttacaccacaatatatctgccca

SEQ ID NO:141 is the nucleic acid sequence of pRD29A-antisense-GmCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the GmCPP antisense sequence.

SEQ ID NO:142

gtttacccgcgaatataatcctgtcaaacactgatagtttaaactgaaggcgaggaaacgacaatc  
 tgatcatgagcggagaattaaggaggtcacgttatgacccccgccgatgacgcgggacaagccg  
 ttttacgtttggaactgacagaaccgcaacgttgaaggagccactcagccgagggtttctggag  
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 atgattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggctattcgggt  
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 gcgcccgggttcttttgtcaagaccgacctgtccgggtgcctgaatgaactgcaggacgaggca  
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SEQ ID NO:142 is the nucleic acid sequence of pBI121-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold is the BnCPP antisense sequence.

SEQ ID NO:143

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SEQ ID NO:143 is the nucleic acid sequence of pBI121-HP-BnCPP. Italicized sequences  
 are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in bold  
 is the BnCPP antisense sequence, bold and underlined sequence is the BnCPP sense fragment  
 and upper case indicates the truncated GUS fragment.

SEQ ID NO:144

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tgcaggatttcttctctggatcgagtcccaccattgggtagaaagccgccagatatcttccaaa  
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gccggtcttgcgatgattatcatataatttctgttgaaattacgttaagcatgtaataattaaca  
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cccgcaccgatcgcccttcccaacagttgcgcagcctgaatggcgcccgctcctttcgctttct  
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 ctactggtgaaaagaaaaaccaccccgtagattaaaaacgtccgcaatgtgttattaagttg  
 tctaagcgtcaatttggtttacaccacaatatatcctgccca

SEQ ID NO:144 is the nucleic acid sequence of pBI121-antisense-BnCPP. *Italicized* sequences are the right and left border repeats. Underlined sequence is the 35S promoter. Sequence in **bold** is the BnCPP antisense sequence.

#### SEQ ID NO:145

*gtttacccgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatc*  
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*ttttacgtttggaactgacagaaccgcaacgttgaaggagccactcagccgcgggtttctggag*  
*tttaatgagctaagcacatacgtcagaaaccattattgcgcgttcaaaagtgcctaagggtcac*  
*tatcagctagcaaatatttcttgtaaaaaatgctccactgacgttcataaattcccctcggt*  
*tccaattagagtctcatattcactctcaatccaaataatctgcaccggatctggatcgtttcgc*  
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*aagcgggaagggactggctgctattgggcgaagtgccggggcaggatctcctgtcatctcacct*  
*tgctcctgccgagaaagtatccatcatggctgatgcaatgcggcgggtgcatacgtttgatccg*  
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*atcgttttccgggacgcgggtggatgatcctccagcgcggggatctcatgctggagttcttcg*

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agcgtcaatttgtttacaccacaatatatcctgccca

SEQ ID NO:145 is the nucleic acid sequence of pRD29A-BnCPP. *Italicized sequences* are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in **bold** is the BnCPP sense sequence.

SEQ ID NO:146

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ACATGGCATCGTGGTGATTGATGAAACTGCTGCTGTCGGCTTTTCGCTCTCTTTAGGCATTGGT  
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 ctgcca

SEQ ID NO:146 is the nucleic acid sequence of pRD29A-HP-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in bold is the BnCPP antisense sequence, bold and underlined sequence is BnCPP sense fragment and the upper case sequence represents the truncated GUS fragment.

SEQ ID NO:147

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SEQ ID NO:147 is the nucleic acid sequence of pRD29A-antisense-BnCPP. *Italicized* sequences are the right and left border repeats. Underlined sequence is the RD29A promoter. Sequence in **bold** is the BnCPP antisense sequence.

SEQ ID NO:148

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 ccacaatatatcctgccca

SEQ ID NO:148 is the nucleic acid sequence of MuA-BnCPP. Italicized sequences are the right and left border repeats. Underlined sequence is the MuA promoter. Sequence in bold is the BnCPP sense sequence.

### Example 33. Southern Analysis

Genomic Southern blot analysis of transgenic *Arabidopsis* was performed using standard techniques known to one skilled in the art. Typically, 10µg of DNA was electrophoresed in a 0.8% agarose gel and transferred to an appropriate membrane such as Hybond N+ (Amersham Pharmacia Biotech). Pre-hybridization and hybridization conditions were as suggested by the membrane manufacturer, typically at 65°C. The final stringency wash was typically at 1XSSC and 0.1% SDS at 65°C. The NPTII coding region was typically used as the radiolabeled probe in Southern blot analysis.



Thirty-seven *Arabidopsis* lines were selected as homozygous pBI121-AtCPP over-expression lines for further examination. Figure 27 shows a representative blot confirming the presence of the pBI121-AtCPP transgene. Lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

Thirty-three *Arabidopsis* lines were selected as homozygous pBI121-HP-AtCPP hair-pin down-regulation lines for further examination. Figure 28 shows a representative blot confirming the presence of the pBI121-HP-AtCPP hair-pin construct. All lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

*Arabidopsis* lines were selected as homozygous pRD29A-AtCPP over-expression lines for further examination. Figure 29 shows a representative blot confirming the presence of the pRD29A-AtCPP transgene. Lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

*Arabidopsis* lines were selected as homozygous pRD29A-HP-AtCPP lines for further examination. Figure 30 shows a representative blot confirming the presence of the pRD29A-HP-AtCPP transgene. Lines were confirmed to be transgenic by PCR analysis using transgene specific primers in the PCR assays.

#### **Example 34: PCR analysis of transgenic plants**

PCR was used as a method to confirm the presence of the transgene in all transgenic lines and every construct. Typical PCR mixtures contained: 1X reaction buffer (10mM Tris-HCl pH 8.8, 1.5mM MgCl<sub>2</sub>, 50mM KCl), dNTP's at 200μM, 1pM forward and reverse primer, 2.5U. *Taq* DNA polymerase, and template plus water to a final volume of 50μL. Reactions were run at 1 minute 94°C, 1 minute 60°C, 1 minute 72°C, for 30 cycles. Primers used in the analysis of pBI121-AtCPP and pBI121-HP-AtCPP transgenic plants were as shown in Table 20. Primers used in the analysis of pRD29A-AtCPP were RD29AP1 (SEQ ID NO:161) and SEQ ID NO:102. Primers used in the analysis of pRD29A-HP-AtCPP transgenic plants were those identified as RD29AP1 (SEQ ID NO:161), SEQ ID NO:103 and SEQ ID NO:103, Nosterm-RV (SEQ ID NO:162).

#### **Table 20.**

pBI121-AtCPP BamFW: 5'-GCCGACAGTGGTCCCAAAGATGG-3'  
(SEQ ID NO:105)

p35S-AtCPP *Sma*RV: 5'-AAACCCGGGTTAATCTGTCTTCTTGTCTTCTCCA-3' (SEQ ID NO:102)

p35S-HP-AtCPP *Bam*FW: 5'-CTGGAGCTCTTTTACCGAGGTTGGGCCTTGATCC-3' (SEQ ID NO:103)

p35S-HP-AtCPP *Sma*RV: 5'-GCAAGACCGGCAACAGGA-3' (SEQ ID NO:108)

pRD29AP1: 5'-TTTAAGCTTGGAGCCATAGATGCAATTCAA -3' (SEQ ID NO:161)

pRD29AP1: 5'-TTTAAGCTTGGAGCCATAGATGCAATTCAA -3' (SEQ ID NO:161)

Nosterm-RV: 5'-GCAAGACCGGCAACAGGA-3' (SEQ ID NO:162)

### Example 35: Northern analysis of transgenic plants

Total RNA was isolated from developing leaf tissue of 27 35S-AtCPP *Arabidopsis* lines (T3 plants). Approximately 10 µg of total RNA was loaded into each lane. The Northern blot was first probed with P<sup>32</sup> labeled, single-stranded antisense transcript of AtCPP which detects sense transcript, then stripped and re-probed with cDNA of β-tubulin that was used as a reference. The hybridizing bands of AtCPP and β-tubulin were scanned and quantified using the UN-Scan-It programme (Silk Scientific, Utah, USA), and the ratio of the two hybridizing bands for each sample was obtained. The ratio of the wild type plants was set to 100%, and was compared with those of the transgenic lines. Twenty-one out of twenty-seven lines showed higher expression of AtCPP transcript as compared to the wild type. Values ranged from 104 % to 282 % of wild type. The results of five lines (35, 84, 76, 136, and 156) of the 21 over-expressing lines is shown in Figure 31.

### Example 36: Production of polyclonal antibodies against AtCPP

Anti-AtCPP antibodies were generated using AtCPP fusion protein over-expressed in *E. coli*. The over-expression vector, pMAL-p2, contains 1175 bp *malE* gene that is located upstream of AtCPP and encodes a 43 KDa maltose-binding protein (MBP). The 1275 bp *Bam*HI/*Sma*I DNA fragment of AtCPP was inserted into pMAL-p2 at *Bam*HI and *Sal*I sites. The *Sal*I site was converted into blunt end using Klenow fragment. The resulting fusion protein

MBP-AtCPP was then over-expressed in DH5 $\alpha$ , and purified by one-step affinity for MBP as described by the manufacturer (New England Biolab). The soluble fraction of the crude bacterial extract containing the MBP-AtCPP fusion protein was loaded to a amylose column (1.5 cm x 10.0 cm), and the proteins were eluted with 10 mM maltose in column buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 200 mM NaCl). Fractions containing purified MBP-AtCPP fusion protein were pooled, and concentrated with a Centriprep-30 concentrator (Amicon). All purification steps were carried out at 4°C. To generate an antibody, the purified fusion protein was further separated by SDS-PAGE and the Coomassie stained band corresponding to the fusion protein was excised. The identity of the fusion protein was confirmed by Western analysis using anti-MBP antibodies (purchased from New England Biolab). The protein was eluted from the gel slice by electroelution and then emulsified in Ribi adjuvant (Ribi Immunochem) to a final volume of 1 ml. MBP-AtCPP protein was injected into a 3 kg New Zealand rabbit on day 1 and booster injections were given on day 21 and day 35 with 175  $\mu$ g of the protein each time. High-titer antisera were obtained one week after the final injection.

**Example 37: Western blot analysis of 35S-AtCPP transgenic lines using Anti-AtCPP antibodies.**

Western analysis was performed to examine expression level of AtCPP in the transgenic lines compared with that of wild type plants. Anti-Bip antibody, an ER luminal protein (Stressgen, Victoria, BC, Canada ) was used as a reference. Total proteins were extracted from developing leaf tissue of five ABA<sup>S</sup> lines and a wild type control.. The antigenic protein bands of AtCPP and Bip were scanned and quantified using the UN-Scan-It programme (Silk Scientific, Utah, USA) and the ratio of the two protein bands for each sample was obtained. The ratio of the wild type plants was set to 100%, and was compared with those of the transgenic lines. Data is presented in Figure 31 indicating that the AtCPP protein level was increased in the transgenic lines compared to the wild type plants.

**Example 38: ABA sensitivity of transgenic seedlings.**

Approximately 100 seeds were assessed per line per 9 cm plate. Seeds were plated on minimal medium (1/2 MS) supplemented with no ABA or 1.0  $\mu$ M ABA. Plates were chilled for 3 days at 4 °C in the dark, and incubated for up to 21 days at 22 °C with 24 hour continuous light. Plates were assessed for germination, cotyledon expansion, true leaf development and seedling vigor. Seedlings were assessed for ABA sensitivity over 21 days of growth at which time sensitive seedlings were arrested at the cotyledon stage, lacked true leaves, and showed

inhibition of root growth. Wild type control Columbia plants had two to three pairs of true leaves and a well developed root system. Lines were categorized as ABA sensitive (ABA<sup>S</sup>) if less than 1% of plants looked like control, moderately ABA sensitive (ABA<sup>MS</sup>) if more than 1% but less than 50% of looked like control, or ABA insensitive (ABA<sup>Wt</sup>) if greater than 50% looked like control.

For example, if a plate had 20 healthy seedlings and the control plate had 60 healthy seedlings, the line would be 33% of control and categorized as moderately ABA sensitive.

All four vector constructs (pBI121-AtCPP, pBI121Hp-AtCPP, pRD29AHp-AtCPP, pRD29A-ATCPP) have resulted in transgenic lines of *Arabidopsis* which have increased sensitivity to ABA which is indicative of stress tolerance. The data for all 4 constructs is shown in Figure 32. Of the lines transformed with the pBI121-AtCPP construct to over-express the AtCPP gene, 58% (21 out of 36) were classified as sensitive and an added 30% (11 out of 36) were classified as moderately sensitive. These lines were tested again in T4 and T5 generations and their ABA sensitivity was still present indicating that ABA sensitivity is an inheritable trait. Of the lines transformed with the pBI121-HP-AtCPP construct to down-regulate the AtCPP gene by double stranded RNA-inhibition, 15% (7 out of 45) were classified as sensitive and 31% (14 out of 45) were classified as moderately sensitive. To illustrate the increased sensitivity of transgenic lines to ABA, Figure 33 shows the results of germination and seedling development over a range of ABA concentrations. Wild type and pRD29A-HP-AtCPP are compared. Of the lines transformed with pRD29AHp-AtCPP 70% (12 out of 17) showed high sensitivity and 24% (4 out of 17) showed moderate sensitivity to ABA. Of the lines transformed with pRD29A-AtCPP 29% (5 out of 17) showed high sensitivity and 12% (2 out of 17) moderate sensitivity to ABA. Clearly all 4 transgene constructs are altering ABA sensitivity and ABA signal transduction.

#### **Example 39: Drought Experiments**

*Arabidopsis* plants were grown five plants per 4" or 3" pot, in a replicated water-stress experiment. All pots were filled with equal amounts of homogeneous premixed and wetted soil. Plants were grown under 16 hour daylight (150-200  $\mu\text{mol}/\text{m}^2/\text{s}$ ) at 22 °C and 70% relative humidity. On the day that the first flower opened drought treatment was initiated. First soil water content in each pot was equalized on a weight basis and any further watering of plants was stopped. Daily measurements of soil water content were taken by recording total pot weight. At the end of the drought treatment (6 to 9 days for experiments in 4" pots and 4-5 days for

experiments in 3" pots) plants were harvested and shoot dry weights determined. Differences in plant growth were factored into the analysis by expressing water loss on a per gram shoot dry weight basis.

### 39a) pBI121-AtCPP, Drought stress screen:

Analysis of pBI121-AtCPP transgenic lines during water-stress treatment experiments of up to an eight day period, shows a strong trend towards increased soil water content and reduced water loss per gram of shoot biomass. After three days of water-stress treatment most lines had increased soil water content relative to the wild type control with four out of twenty-four lines, 146, 149, 156 and 97, showing a statistically significant difference. The amount of water lost per gram of shoot biomass was lower for all lines except one (95), and thirteen of these lines were significantly different from the wild type Columbia control (Figure 34). All of the lines showing a statistically significant lower water loss per gram shoot biomass also showed an increased ABA sensitivity. There is also a strong trend, for all but one line (95), which is ABA<sup>wt</sup>, towards greater shoot biomass at the end of the drought stress treatment. Seven of those lines 136, 146, 23, 46, 76, 84 and 9, were statistically significant from control at a p=0.05 value.

### 39b) pBI121-AtCPP, Water loss per gram shoot biomass during water stress treatment:

Lines 35, 76, 95 and a wild type control were grown and placed under a water-stress treatment as above. Plants were harvested at 2 days, 4 days and 6 days of drought treatment. The ABA<sup>S</sup> lines, 35 and 76, showed a statistically significant reduction in water-loss relative to shoot dry weight at all three time points (Table 21). Additionally, the two ABA<sup>S</sup> transgenic lines had increased shoot biomass, due to increased leaf biomass, and maintained higher soil water contents during drought treatment.

**Table 21.** Water loss (g) per Shoot dry weight (g) after 2, 4 and 6 days of drought-stress treatment. Values in bold indicate statistically significant differences from Columbia.

Line	2 days		4 days		6 days	
	Mean	Std. Error	Mean	Std. Error	Mean	Std. Error
35	<b>212.5</b>	3.5	<b>308.0</b>	9.9	<b>297.7</b>	11.2
76	<b>227.2</b>	5.8	<b>321.2</b>	8.5	<b>293.8</b>	5.0
95	287.0	5.1	<b>377.3</b>	14.8	348.5	25.5
Columbia	265.3	11.8	408.2	7.7	345.9	6.7

Wild type						
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### 39c) pBI121-AtCPP, Drought stress and shoot recovery:

Water-stress tolerance and determination of post drought-treatment recovery ability was assessed using 20 of the 24 pBI121-AtCPP transgenic lines. Drought treatment was imposed for 6 days after which the plants were watered and allowed to grow for 6 days. Recovered shoot fresh biomass was then determined. Soil water content of these plants was measured daily during the drought treatment and the results confirm previously seen trends. All ABA sensitive (ABA<sup>S</sup>) lines that showed a statistically significant reduction of water loss on a per gram dry weight basis in experiment 39a, continued to show a significant greater soil water content than control plants in this experiment (Table 22). Additionally, Table 22 shows that the recovered shoot fresh biomass after 6 days of drought treatment was significantly greater in all the ABAs lines than Columbia.

**Table22.** Soil water content on day 3 of drought treatment and recovered shoot fresh weight after 6 days of drought treatment (values in bold were significantly different from Columbia at p=0.05)

	ABA status	soil water content day 3		recovered shoot biomass	
Line	ABA	Mean (%) initial)	Std Error	Mean (g)	Std Error
136	ABA <sup>S</sup>	<b>46.6</b>	1.9	<b>4.5</b>	0.16
14	ABA <sup>S</sup>	<b>50.25</b>	0.7	<b>4.1</b>	0.12
146	ABA <sup>S</sup>	<b>45.9</b>	2.5	<b>4.0</b>	0.11
147	ABA <sup>S</sup>	45.1	1.7	<b>4.0</b>	0.15
149	ABA <sup>S</sup>	45.3	1.8	<b>3.8</b>	0.17
156	ABA <sup>S</sup>	<b>47.1</b>	1.9	<b>4.0</b>	0.134
23	ABA <sup>S</sup>	<b>49</b>	1.4	<b>4.0</b>	0.17
33	ABA <sup>S</sup>	<b>46.9</b>	1.6	<b>4.3</b>	0.14
35	ABA <sup>S</sup>	41.7	1.7	<b>4.0</b>	0.11

46	ABA <sup>S</sup>	44.8	1.7	3.8	0.09
63	ABA <sup>S</sup>	46.3	1.4	4.0	0.19
76	ABA <sup>S</sup>	47.8	1.0	3.9	0.17
79	ABA <sup>S</sup>	45.4	1.1	4.1	0.09
84	ABA <sup>S</sup>	46.8	1.9	4.1	0.16
85	ABA <sup>S</sup>	45.3	1.9	4.0	0.12
9	ABA <sup>S</sup>	45.2	2.1	3.9	0.12
93	ABA <sup>wt</sup>	43.5	1.2	2.8	0.07
94	ABA <sup>S</sup>	46.9	1.5	3.9	0.13
97	ABA <sup>S</sup>	53	1.2	3.8	0.16
95	ABA <sup>wt</sup>	41.9	1.2	2.7	0.06
Columbia	ABA <sup>wt</sup>	41.3	1.0	2.7	0.04

**39d) pBI121-AtCPP, Seed yield after drought stress treatment:**

Seed yield after drought stress during flowering was examined using ten pBI121-AtCPP transgenic lines, eight of which were ABA<sup>S</sup>. Plants were grown one per 4" pot and were exposed to 9 days of drought treatment as described above. A second group of plants was grown and maintained under well watered conditions as the optimal group. After 9 days of drought treatment plants were re-watered and allowed to continue growth and seed set to maturity. After drought-treatment conditions all eight ABA<sup>S</sup> lines had increased yields relative to controls, which ranged from 109% to 126% of the Columbia (Table 23). Drought-treatment resulted in a reduction of yield in all lines, including controls, relative to plants grown under optimal conditions. Expression of the seed yields obtained from drought-treated group relative to the same line under optimal conditions shows that the transgenics preserve a larger percentage of optimal seed yield than do wild type lines.

**Table 23.** Seed Yield following 9 days drought-treatment

	ABA status	Seed Yield (g per plant)			
Line	ABA	Mean (g)	Std Error	% Columbia	% Optimal
156	ABA <sup>S</sup>	0.735	0.044	126.2	83.7
63	ABA <sup>S</sup>	0.675	0.061	116.0	71.0
146	ABA <sup>S</sup>	0.666	0.053	114.4	72.9
94	ABA <sup>S</sup>	0.644	0.052	110.6	68.8
84	ABA <sup>S</sup>	0.642	0.049	110.4	61.8
76	ABA <sup>S</sup>	0.631	0.055	108.5	66.6
136	ABA <sup>S</sup>	0.630	0.051	108.3	74.1
35	ABA <sup>S</sup>	0.614	0.054	105.6	74.2
93	ABA <sup>wt</sup>	0.567	0.041	97.5	60.0
95	ABA <sup>wt</sup>	0.388	0.088	66.7	43.4
Columbia	ABA <sup>wt</sup>	0.582	0.060	100	53.8

### 39e) pBI121-AtCPP, Seed yield and growth under optimal water conditions:

The lines evaluated above and a number of additional lines were examined in a growth and yield experiment under optimal, well-watered conditions. Results indicated that the ABA<sup>S</sup> lines were shorter at the stage of first open flower, had more rosette leaves, however, by maturity there were no differences in plant height of transgenics and Columbia. Moreover, the ABA<sup>S</sup> transgenics showed similar or higher seed yields ranging from 95% to 121% of the wild type control (Figure 35).

### 39g) pRD29A-HP-AtCPP screen for drought tolerant phenotype:

Analysis of 17 transgenic lines identified 7 candidate drought tolerant lines (12, 22, 23, 47, 82, 83, 90) on the basis of higher soil water content and lower water loss per g of shoot dry weight (Table24). All 7 drought tolerant candidate lines showed strong ABA sensitivity and lines that did not show drought tolerance did not show ABA sensitivity.



**Table 24.** Soil water content after 3 days of drought treatment and water lost per g shoot dry weight. Values in bold are statistically different from those of Columbia wild type (p=0.05)

	ABA status	soil water content day 2		water lost in 2days/g shootDW	
Line	ABA	Mean (%) initial)	Std Error	Mean (g/g)	Std Error
10	ABA <sup>S</sup>	33.4	1.6	199.1	4.5
11	ABA <sup>S</sup>	34.6	3.3	<b>173.1</b>	1.6
12	ABA <sup>S</sup>	36.2	2.0	179.5	5.0
126	ABA <sup>MS</sup>	32.5	2.6	199.1	4.1
127	ABA <sup>MS</sup>	33.5	2.0	195.6	10.6
14	ABA <sup>S</sup>	32.7	1.2	203	4.9
17	ABA <sup>S</sup>	29.9	1.8	200.7	7.3
22	ABA <sup>S</sup>	<b>39.3</b>	2.1	<b>170.0</b>	3.0
23	ABA <sup>S</sup>	35.7	1.4	<b>174.9</b>	2.6
42	ABA <sup>MS</sup>	28	0.7	185.4	5.8
47	ABA <sup>S</sup>	35.9	2.2	181.2	7.7
7	ABA <sup>Wt</sup>	35	1.3	201.8	5.1
82	ABA <sup>S</sup>	36.7	2.2	178.3	4.0
83	ABA <sup>S</sup>	<b>40</b>	1.4	180.7	6.9
9	ABA <sup>S</sup>	31.4	1.4	173.8	8.7
90	ABA <sup>S</sup>	<b>38.2</b>	1.3	<b>177.6</b>	6.2
93	ABA <sup>Wt</sup>	30.7	1.8	175.3	4.6
Columbia	ABA <sup>Wt</sup>	32.1	1.2	196.9	6.2

**Example 40. Growth Analysis**

The growth analysis of most promising constructs has been set up at 3 stages. Eight plants per line were grown in 3" pots with one plant per pot at 22C, 16hr light (150-200  $\mu\text{mol/m}^2/\text{s}$ ) and 70% RH. Plants were harvested at vegetative growth stage (2 week old seedlings), bolting growth stage (at first open flower) and mid-flowering growth stage (5 to 7 days from first open flower). Also, in some growth experiments additional group of plants was grown in 4" pots (one per pot and 10 plants per line) to maturity for seed yield determinations.

**40a) pBI121-AtCPP growth under optimal and biotic stress conditions**

The growth and productivity of pBI121-AtCPP transgenic *Arabidopsis* lines was examined at several stages of development under optimal growth conditions. Although optimal growth conditions were maintained, plants were assessed to be under a degree of stress that was later determined to be a result of the soil properties. Soil analysis found a fungal contaminant that was believed to be responsible for the biotic stress. This stress could be negated by sterilization of the soil prior to use. Eight ABA<sup>S</sup> lines, two with normal ABA sensitivity (ABA<sup>Wt</sup>) and a wild type Columbia control were analyzed.

Figure 36 presents the results of various growth (from mid-flowering stage) and yield parameters and each trait is expressed as a percentage of the Columbia control. The results strongly support an enhanced growth phenotype. This enhanced growth phenotype is present at all growth stages. At the vegetative stage, all ABA<sup>S</sup> transgenic plants showed an increase in leaf number relative to that of the wild type with four of the eight lines showing a statistically significant difference. The two ABA<sup>Wt</sup> lines showed the same or fewer leaves relative to wild type.

At the bolting stage ABA<sup>S</sup> transgenics showed an increase in leaf number but plants were shorter at this stage (first open flower) than controls. The shoot fresh weight of transgenics was significantly increased relative to that of controls, ranging from 80% to 342% of the wild type. The ABA<sup>S</sup> transgenics displayed a delay in flowering from one to three days. The ABA<sup>Wt</sup> transgenics did not show delayed flowering, increased shoot fresh weight or increased height.

At the flowering stage of development the enhanced growth phenotype is maintained (greater leaf number and fresh weight), however, there were no observable differences in plant height indicating that transgenics bolt shorter but reach same final plant height.

Of particular significance is the observation, that under these conditions (biotic stress due to presence of fungi in the soil) yields of the ABA<sup>S</sup> transgenics were significantly higher, ranging from 120% to 229% of the wild type control. The ABA<sup>Wt</sup> lines showed similar or

slightly reduced yields relative to the Columbia control. This finding indicates that ABA<sup>S</sup> transgenic lines are affected less by the biotic stress. This observation has been confirmed, where 5 of the drought tolerant lines were grown in contaminated soil to maturity. The seed yields of transgenic lines, even though greatly reduced relative to optimal conditions, were 2.5 to 4.5 fold higher than those of Columbia wild type (Table 25).

**Table 25.** Seed yield of pBI121-AtCPP lines grown in contaminated soil. Values in bold indicate statistical differences at p=0.05

Line	ABA sensitivity	Seed Yield per plant (g)	% of Columbia
156	ABA <sup>S</sup>	<b>0.33 ± 0.04</b>	316%
23	ABA <sup>S</sup>	<b>0.35 ± 0.05</b>	336%
76	ABA <sup>S</sup>	<b>0.31 ± 0.04</b>	296%
84	ABA <sup>S</sup>	<b>0.25 ± 0.33</b>	237%
9	ABA <sup>S</sup>	<b>0.48 ± 0.05</b>	455%
Columbia	ABA <sup>wt</sup>	0.11 ± 0.03	

#### 40b) pBI121-AtCPP early seedling growth:

Four ABA<sup>S</sup> and one ABA<sup>wt</sup> line plus Columbia were examined for early seedling growth on agar plates. Twenty seeds were plated in a line on agar plates containing 50% MS with 1% sucrose and vitamins and 6 plates per line were used. Plates were placed on slants, which allowed roots to grow downwards. Root length was measured on 7-day old seedlings and shoot and root biomass determined on 11-day old seedlings. Two of the ABA<sup>S</sup> transgenic lines had significantly longer roots and all 4 ABA<sup>S</sup> lines had shoot dry weights 114% to 123% of controls and root dry weights of 116% to 151% of controls. As a result, the shoot biomass to root biomass ratios were slightly reduced in transgenics. These results indicate that enhanced growth of these transgenics is evident in the early growth stage, shortly after germination, and the root growth is more enhanced relative to shoot growth. In a different experiment seedlings were pulled out of agar and roots were stained with toluidine blue to show their structure. Figure 13 shows that transgenic lines had more extensive lateral root system, which would account for greater root biomass.

**40c) pRD29A-HP-AtCPP optimal growth characteristics**

An optimal growth study has been conducted with 10 lines as described before. Vegetative growth data showed that two of the lines (12 and 9) had significantly more leaves and seven of the lines (12, 22, 23, 47, 82, 9) had significantly greater shoot biomass. Bolting data showed that eight of the lines (12, 22, 23, 47, 82, 9, 90, 93) were significantly delayed in flowering by one to two days, and seven of the lines were significantly shorter than Columbia at first open flower. All of the lines except 42 and 7 had significantly greater number of rosette leaves and shoot FW and this trend is maintained into the mid-flowering harvest (Figure 38). The plant height, however, by mid-flowering harvest was not significantly different between the transgenic lines and control. All the lines that showed this enhanced growth also showed drought tolerance and ABA sensitivity.

**Example 41. Ultrastructure pBI121-AtCPP**

Two of the drought tolerant and ABA<sup>S</sup> lines (35 and 76) plus Wt Columbia were used to examine stem and root cross-sections for any differences in ultrastructure. Free hand sections of mature stems (plants flowering for 10 days) were obtained from above the first node, stained with toluidine blue and preserved with glycerol. The stems of transgenic plants appeared to have more dense cellular structure and contain one or two more vascular bundles than those of Columbia Wt indicating more enhanced water and nutrient transport system.

Leaf disks were taken and fresh weights determined. Transgenic leaf disks were significantly heavier, 20-24% greater than corresponding wild type controls. This increase is believed to be as a result of a thicker leaf.

**Example 42. Cold stress experiment pBI121-AtCPP**

Four drought tolerant, ABA<sup>S</sup> lines (156, 23, 35, 76) and one ABA<sup>Wt</sup>(95) line plus wild type Columbia were included in a cold stress study. Plants were grown in 3" pots one per pot) with 10 replicate pots per line at 22C for 10 days (7 days on agar plates and 4 in soil). The cold stress group was moved into 7°C for 5 days while the optimal group was left at 22C. After 5 days in the cold both cold stress group and the optimal group were harvested for shoot biomass determination. ABA<sup>S</sup> and drought tolerant lines had significantly greater shoot biomass than Columbia in both optimal (25 to 39% greater shoot fresh weight) and cold stress groups (18 to 44% greater shoot DW) (Table 26). Results of an eight-day cold stress showed that differences between the transgenic lines and Columbia were even more pronounced (53 to 61% greater

shoot fresh weight). This result indicates greater plant vigor and better ability of transgenics to cope with cold stress.

**Table 26.** Shoot fresh weight of optimal and cold stressed (5C for 5d) pBI121-AtCPP. Values in bold indicate statistical difference at  $p=0.05$

Line	ABA sensitivity	Optimal shoot FW		Cold stress shoot FW	
		mg	% of Columbia	mg	% of Columbia
156	ABA <sup>S</sup>	<b>95.4 ± 3.7</b>	137%	<b>23.1 0.7</b>	118%
23	ABA <sup>S</sup>	<b>96.3 ± 3.9</b>	139%	<b>28.3 1.5</b>	144%
35	ABA <sup>S</sup>	<b>87.0 ± 1.7</b>	125%	<b>25.3 1.4</b>	130%
76	ABA <sup>S</sup>	<b>94.7 ± 2.2</b>	136%	<b>27.3 1.5</b>	140%
95	ABAWt	67 ± 2.4	96%	21.4 1.0	109%
Columbia	ABAWt	69 ± 1.9		19.6 1.1	

**Example 43. Drought stress under high temperature pBI121-AtCPP**

A drought stress experiment was conducted as described above except that day temperature of 32°C (16hr) and night temperature of 22°C (8hr) was maintained. These temperatures were achieved daily over a 2hr ramping period. Four ABA<sup>S</sup> and one ABA<sup>Wt</sup> line plus Columbia were included. Plants were monitored daily for water loss and soil water content and after 5 days of drought treatment half of the plants were harvested and the other half was re-watered and allowed to recover for four days. Shoots were harvested and shoot fresh weight determined. The results (Table 27) of this experiment showed that previously identified drought tolerant lines maintained their drought tolerant phenotype at high temperature and were able to recover well from the drought stress at high temperature

**Table 27.** Soil water content on day 2 and water lost in 2 days/final shoot dry weight plus recovery shoot FW after 5days of drought stress at 32C day and 22C night temperatures. Values in bold indicate significant differences from the Columbia control.

line	ABA sensitivity	soil water content day 2	water lost in 2d/shoot DW	recovered shoot FW (g)
136	ABA <sup>S</sup>	50.4 ± 1.1	<b>485.7 ± 18.5</b>	<b>1.30 ± 0.04</b>
146	ABA <sup>S</sup>	<b>52.1 ± 1.0</b>	<b>504.5 ± 7.9</b>	<b>1.15 ± 0.04</b>
35	ABA <sup>S</sup>	<b>52.2 ± 0.8</b>	<b>502.8 ± 15.8</b>	<b>1.19 ± 0.02</b>
76	ABA <sup>S</sup>	<b>52.1 ± 0.6</b>	<b>435.6 ± 10.5</b>	<b>1.11 ± 0.03</b>
95	ABAWt	50.0 ± 0.9	518.2 ± 13.0	0.86 ± 0.03
Columbia	ABAWt	48.6 ± 0.6	559.7 ± 19.0	0.84 ± 0.03

**Example 44. Heat stress and seed yield pBI121-AtCPP**

Two ABA<sup>S</sup> lines and one ABA<sup>Wt</sup> line plus Columbia were examined for the effect of heat stress during flowering on the final seed yield. Plants were grown in 4 inch pots (one/pot) as described above and 9 days from first open flower the temperature was ramped from 22 C to 43C over 2 hours and plants were kept at 43C for 2hr. Temperature was then ramped back to 22C over 2 hours and plants were grown under optimal conditions until maturity. The seed yields from this experiment are shown in Table 28. One of the drought tolerant lines (35) had significantly greater yield than Columbia.

**Table 28.** Seed yield of pBI121-AtCPP lines after two hour 43C heat stress 9 days from first open flower. Values in bold are statistically significant from Columbia.

Line	ABA sensitivity	seed yield (g/plant)	seed yield (% of col.)
35	ABA <sup>S</sup>	<b>0.55 ± 0.05</b>	347%
76	ABA <sup>S</sup>	0.24 ± 0.03	148%
95	ABAWt	0.11 ± 0.02	69%
Columbia	ABAWt	0.16 ± 0.03	

The effect of heat shock on lines of pBI121-AtCPP at the early flowering stage was assessed. Three ABA<sup>S</sup> lines (76, 136, 97) a ABA<sup>Wt</sup> line (95) and a Columbia wild type control

were seeded in 128 cell flats, one flat per line. At the early flowering stage flats were exposed to a temperature of 46.8°C for 50 minutes and then returned to normal growth conditions. Lack of continued growth from main meristems was defined as main meristem death and scored for each line. Data is shown in Table 29.

**Table 29.** Meristem death due to heat shock

Line	<u>Wt</u>	<u>95</u>	<u>76</u>	<u>136</u>	<u>97</u>
% Death	91	97	79	59	18

**Example 45. Stomata density determinations pBI121AtCPP**

Two ABA<sup>S</sup> lines (76 and 35) plus Columbia were examined for stomata density on the upper and lower leaf surface. Nail polish imprints of the upper and lower epidermis were obtained from a fully expanded leaf #5. These imprints were analyzed under the microscope and the number of stomata per  $8.7 \times 10^{-8} \text{ m}^2$  were counted. There were no significant differences found between transgenics and Columbia in the stomata of the upper or lower epidermis (Table 30). The increases seen in drought tolerance and reduced water loss is not attributable to a reduced number of leaf stomata.

**Table 30.** Stomata numbers per  $8.7 \times 10^{-8} \text{ m}^2$  of abaxial and adaxial epidermis of fully expanded leaf #5 in pBI121AtCPP.

Line	ABA sensitivity	stomata on upper epidermis	stomata on lower epidermis
35	ABA <sup>S</sup>	$68 \pm 5$	$103 \pm 7$
76	ABA <sup>S</sup>	$58 \pm 6$	$120 \pm 16$
Columbia	ABAWt	$57 \pm 6$	$116 \pm 11$

**Example 46. CPP Consensus Sequences**

Also included in the invention is the CPP consensus sequences. The consensus sequences were generated by alignment of the CPP polypeptide and nucleic acid sequences as well as sequences homologous using the program BioEdit.

The “x” in the consensus sequence represents any amino acid or nucleotide. Preferably “x” a conservative amino acid or nucleotide substitution. More preferably, “x” is the most amino acid or nucleotide most prevalent at a given postion. For example, the amino acid at postion 145 of SEQ ID NO: 168 is a proline as it occurs 66% of the time.

**Table 31. ClustalW Analysis of BASF Nucleic Acids**

1) <b>BASF_AT1</b>	(SEQ ID NO:116)
2) <b>BASF_AT2</b>	(SEQ ID NO:118)
3) <b>BASF-Corn</b>	(SEQ ID NO:120)
4) <b>BASF-Soy</b>	(SEQ ID NO:122)
5) <b>Consensus</b>	(SEQ ID NO:163)

	10	20	30	40	50	60	
BASF_AT1	..... ..... ..... ..... ..... ..... .....	1					
BASF_AT2	..... ..... ..... ..... ..... ..... .....	1					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	CTAATACGACTCACTATAGGGCAAGCAGTGGTAACAACGACAGTACGCGGGGGGAGACG	60					
Consensus	XX	60					

	70	80	90	100	110	120	
BASF_AT1	..... ..... ..... ..... ..... ..... .....	1					
BASF_AT2	..... ..... ..... ..... ..... ..... .....	1					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	CATGGTTCTGAACAAATTGTTATAAAATAACCTAAAATTTTGAGTTGTCTCTAAACATTG	120					
Consensus	XX	120					

	130	140	150	160	170	180	
BASF_AT1	..... ..... ..... ..... ..... ..... .....	1					
BASF_AT2	..... ..... ..... ..... ..... ..... .....	1					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	GGGTTTAAACAAATCCAATCTCTCAATATAAAACCAATGATCTCACCTCACTCCGTTT	180					
Consensus	XX	180					

	190	200	210	220	230	240	
BASF_AT1	..... ..... ..... ..... ..... ..... .....	8					
BASF_AT2	..... ..... ..... ..... ..... ..... .....	8					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	CTGATTTCTCACTCTTCGTTTCTCGTTCGGTTTCATCAGCGTGTGTCTCAGCCATGGCGTT	240					
Consensus	XX	240					

	250	260	270	280	290	300	
BASF_AT1	TCCCTTCATGGAAACCGTCGTCGGTTTATGATAGTGTGTACATTTTGAGACGTAATTT	68					
BASF_AT2	TCCCTTCATGGAAACCGTCGTCGGTTTATGATAGTGTGTACATTTTGAGACGTAATTT	68					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	TCCCTTCATGGAAACCGTTGTCTGGATTATGATATTAATGTACATTTTGAAACTTACTT	300					
Consensus	XX	300					

	310	320	330	340	350	360	
BASF_AT1	GGATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCGAAAACCTTGGTTGGTGT	128					
BASF_AT2	GGATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCGAAAACCTTGGTTGGTGT	128					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	GGATCTGCGACAAGATAGGGCTCTCAAACTTCTCTTCCAAAGACTTTAGAAAGGTGT	360					
Consensus	XX	360					

	370	380	390	400	410	420	
BASF_AT1	AATTAGCCAAGAGAAAGTTTGAGAAATCACGAGCATACAGTCTTGACAAAAGCTATTTTCA	188					
BASF_AT2	AATTAGCCAAGAGAAAGTTTGAGAAATCACGAGCATACAGTCTTGACAAAAGCTATTTTCA	188					
BASF-Corn	..... ..... ..... ..... ..... ..... .....	1					
BASF-Soy	TATCAGCCAAGAGAAATTTGAGAAATCTAGAGCTATAGTCTTGATAAAAGCACTTCCA	420					



Consensus XXX 420

430 440 450 460 470 480

BASF\_AT1 CTTTGTTCAGAGTTTGTAACTATACTATGACTCTGCAATTTTGTTCCTTGGGATCTT 248

BASF\_AT2 CTTTGTTCAGAGTTTGTAACTATACTATGACTCTGCAATTTTGTTCCTTGGGATCTT 248

BASF-Corn ----- 1

BASF-Soy TTTTGTTCAGAGTTTGTAACTATACTATGACTCTGCAATTTTGTTCCTTGGGATCTT 480

Consensus XXX 480

490 500 510 520 530 540

BASF\_AT1 GCCTTGGTTTTGGAAGATGCTCTGGAGCTGTTTACGAGGTTGGGCCTTCATCCAGAGAA 308

BASF\_AT2 GCCTTGGTTTTGGAAGATGCTCTGGAGCAGTTTACGAGGTTGGGCCTTCATCCAGAGAA 308

BASF-Corn ----- ACCAGCCTGAGTCTGAGAA 20

BASF-Soy GCCC TGGTTTTGGAAGAAATCAGGAGATTTTATGACAAATAGCTGCTTTCAATGCTGAGAA 540

Consensus XXTXXXTXCCXGAGAA 540

550 560 570 580 590 600

BASF\_AT1 TGAATACTGCATACTCTTTCATTCTTGGCTGGTGTATGACATGGTCACACATCACTGA 368

BASF\_AT2 TGAATACTGCATACTCTTTCATTCTTGGCTGGTGTATGACATGGTCACAGATCACTGA 368

BASF-Corn TGAGATAATACACACCTTGCCTTCTTAGCTGGTCCATGTTTGGTCCGAGATTACAGA 80

BASF-Soy TGAATACTGCATACCCTTGCCTTCTTAGCAGGGCTCATGATTGGTCACAGATAACAGA 600

Consensus TGAXATAATXCAAXACCTTXXCTTCTTGGCGXXXATGXXXTTGGTCCXAXATXACXGA 600

610 620 630 640 650 660

BASF\_AT1 TTTGCCATTTTCTTGTACTCAACTTTCTGTATCGAGTCTCGGCATGGGTTCAACAAACA 428

BASF\_AT2 TTTGCCATTTTCTTGTACTCAACTTTCTGTATCGAGTCTCGGCATGGGTTCAACAAACA 428

BASF-Corn CTGCGGTTCTCTCTTCAACTTTTGTATAGAGGCTCGACATGGTTTAAACAAGCA 140

BASF-Soy TTTGCCCTTTTCTTGTACTCAACTTTGTGATTGAGGCCGTCATGGTTTAAATAAGCA 660

Consensus XTGGCCXTTCTTXXTAXTCAACTTTXGTATXGAGXXCCXCATGGXTTAAAXAACA 660

670 680 690 700 710 720

BASF\_AT1 AACAAATATGGATGTTTCATTAGGGACATGATCAAAGGAACATTCTCTCTCTCATACTAGG 488

BASF\_AT2 AACAAATATGGATGTTTCATTAGGGACATGATCAAAGGAACATTCTCTCTCTCATACTAGG 488

BASF-Corn AACATATATGGCTCTTTCATTAGGGATATGATCAAAGGAATTTTACTATCCATGATATTGGG 200

BASF-Soy AACACCATGGCTTATCTTTAGGGACATCCTTAAAGGAATTTCTCTTCTTAATAATTGG 720

Consensus AACXXXATGGXTTCTXTTAGGGAXATGXTAAAGGAAXXTTCTCTCTXATXATXAGG 720

730 740 750 760 770 780

BASF\_AT1 CCCACCATTTGTTGCCGGATAATTTTCATAGTCCAGAAAGGAGGTCCTTATCTTGCCAT 548

BASF\_AT2 CCCACCATTTGTTGCCGGATAATTTTCATAGTCCAGAAAGGAGGTCCTTATCTTGCCAT 548

BASF-Corn CCCACCAATCTGGCTGCTATCATCTACATAGTACAGATTGGAGACCTTACCTGGCTAT 260

BASF-Soy TCCACCATTTGTTGCCGGATCATTTGTAATAGTACAGAAAGGAGGTCCTTATCTTGCCAT 780

Consensus XCCACCATTTCTXGCGXGATXATXXXATAGTCCAGAXXGAGGXCCTTAXXTXGCKAT 780

790 800 810 820 830 840

BASF\_AT1 CTATCTGTGGCAATTCATGTTTATCCTCTCTCTAGTGATGATGACTATATACCCGGTCTT 608

BASF\_AT2 CTATCTGTGGCAATTCATGTTTATCCTCTCTCTAGTGATGATGACTATATACCCGGTCTT 608

BASF-Corn ATATCTGTGGGTTTATGTTTCTATTAGCTCTATGATGATGACAATATACCCGATCTT 320

BASF-Soy CTATCTTTGGGTTTATGTTTGGTCTTCTATTGTGATGATGACCTTTATCCAGTACT 840

Consensus XTATCTTTGGGXTTXXGTTTXXXXTXXCTTXXXTGATGATGACXXTXXCCXXTXXT 840

850 860 870 880 890 900

BASF\_AT1 GATAGACCGCTCTTCAACAAGTTCACCTCTTCCAGATGGAGACCTCCGGGAGAAAT 668

BASF\_AT2 GATAGACCGCTCTTCAACAAGTTCACCTCTTCCAGATGGAGACCTCCGGGAGAAAT 668

BASF-Corn GATAGCTCCCTCTTCAACAAGTTCACCTCTTCCAGAGGATCTCAGGGAAAAAAT 380

BASF-Soy AATAGCTCCACTCTTCAATAAGTTCACTCCACTTCCAGATGGTCACTCAGGGAGAAAT 900

Consensus XATAGGXCCXCTTTTCAAAAGTTCACCTCCXGAXGXXXXCTCXGGGAXAAXAT 900

910 920 930 940 950 960

BASF\_AT1 TGAGAACTTGCTTCTTCTTAAAGTTTCCCTTGAAGAAGCTGTTTGTGTGATGGATC 728

BASF\_AT2 TGAGAACTTGCTTCTTCTTAAAGTTTCCCTTGAAGAAGCTGTTTGTGTGATGGATC 728

BASF-Corn AGAGAACTTGGCAGCTTCCCTCAAGTTTCCCTTGAAGAAAGCTTTTCTGTGATGGATC 440

BASF-Soy CGAGAACTTGCTTCTTCCCTCAACTATCCCTTAAAGAACTATTGTTGTGATGGATC 960

Consensus XGAGAACTTGCCXXXTCTXAAXTTCCCTTAAAXAAXCTTXXGTXXGATGGATC 960

970 980 990 1000 1010 1020

BASF\_AT1 TACAAGTCAAGCCATAGCAATGCTTACATGTATGGTTTCTTTAAGAACAAGGATTGT 788

BASF_AT2	TACAAGGTC AAGCCATAGCAATGCTTACATGTATGGTTTCTTTAAGAACAAGGATTGT	788
BASF-Corn	TACCAAGATCAAGCCACAGTAATGCTTACATGTATGGTTTCTTCAAGAACAAGGCTTATGT	500
BASF-Soy	CACAAGATCAAGTCAAGCAATGCTTATGTATGGATTCTTCAAGAACAAGGATTGT	1020
Consensus	XACXACXTC AAGXCAXAGXAATGCTTAXATGTATGGXTTCTTCAAGAACAAXXGATXGT	1020
	1030 1040 1050 1060 1070 1080	
BASF_AT1	TCTTTATGATACGTTGATTTCAGCAGTGCAAGAAATGAGGATGAAATGTGCGCGTTATTGC	848
BASF_AT2	TCTTTATGATACGTTGATTTCAGCAGTGCAAGAAATGAGGATGAAATGTGCGCGTTATTGC	848
BASF-Corn	ACTCTATGACACATTGATTTCAGCAGTGTAAGAAATGAGGATGAGATAGTTTCTGTTATAGC	560
BASF-Soy	CCTTTATGACACATTGATTTCAGCAGTGCAAGAAATGAGGATGAGAAATGTGCTGTTATTGC	1080
Consensus	XCTTATGAXAAXTTXATTCACAGTGXXXXXAGXGAXGAXATXGTXXCXGTTATTXGC	1080
	1090 1100 1110 1120 1130 1140	
BASF_AT1	ACACGAGCTTGGACATTGGAACCTGAATCACTACATACTCGTTTCATTGCACTTCAAAAT	908
BASF_AT2	ACACGAGCTTGGACATTGGAACCTGAATCACTACATACTCGTTTCATTGCACTTCAAAAT	908
BASF-Corn	ACATGAACCTTGGACACTGGAACCTCAATCATACTGTCTATTCCTTTGTAGCTGTCCAGCT	620
BASF-Soy	CCATGAGTTGGACACTGGAAGCTCAACCACTACTGTGTACACATTGTTGCTATGCAAT	1140
Consensus	XCAXGAXXTXGGACAXTGAAXCTXAXXCAACTXXXATXAXCTTXXTXGXXTXCAXXT	1140
	1150 1160 1170 1180 1190 1200	
BASF_AT1	CCTTCCCTTCTTACAATTTGGAGGATACACTCTTGTTCAGAACTCCACTGATCTCTTCAG	968
BASF_AT2	CCTTCCCTTCTTACAATTTGGAGGATACACTCTTGTTCAGAACTCCACTGATCTCTTCAG	968
BASF-Corn	GCTTATCTTCTTCAATTTGGAGGATATACTCTAGTAAGGAGCTCCAAAGATCTATTGG	680
BASF-Soy	TCTTACACTCTTACAATTTGGAGGATATACACTAGTGCAGAAATTCAGCTGATCTCTATCG	1200
Consensus	XCTTXXXXXXXTXCAATTTGGAGGATAXACXCTXSTXXGAXXTXGXXXXGATCTXTXXG	1200
	1210 1220 1230 1240 1250 1260	
BASF_AT1	GAGTTTCGGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTTCAGCACACTGT	1028
BASF_AT2	GAGTTTCGGATTTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTTCAGCACACTGT	1028
BASF-Corn	AAGTTTTCGGCTTCAAGGACGAGCAATTAATTTGGAATTGATCATTTTCGCGACACAT	740
BASF-Soy	AAGCTTTGGCTTTGATACGAGCCAGTCCTCATTGGCTTCATCATATTTTCAGCATACTGT	1260
Consensus	XAGXTTXGXTTXXAXXXXCAGCCXGTXXTXATTTGXXXTXATCATXTTXXGXCAXACXXT	1260
	1270 1280 1290 1300 1310 1320	
BASF_AT1	AATACCACTCCAACATCCAGTAAGCTTTGGCCTCAACCTTGTTCAGTCCGAGCGTTTGAGTT	1088
BASF_AT2	AATACCACTCCAACATCCAGTAAGCTTTGGCCTCAACCTTGTTCAGTCCGAGCGTTTGAGTT	1088
BASF-Corn	AATACCCATCCAACACCTTCTGAGCTTTTCGCTGAACCTTGTTCAGCAGAGCATTTGAATT	800
BASF-Soy	TAACTTTCAGCAATTCGTCAGCTTTGGTCTGAACCTAGTCAGCCGATCATTGAATT	1320
Consensus	AATXCCXXTXCAXCAXXXXTXAGCTTTXGCTXACCTXGTXAGXXGAXCXTTTGAXTT	1320
	1330 1340 1350 1360 1370 1380	
BASF_AT1	TCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAATCTTCGTCCTACTCTAGT	1148
BASF_AT2	TCAGGCTGATGCTTTTGCTGTGAAGCTTGGCTATGCAAAAATCTTCGTCCTGCTCTAGT	1148
BASF-Corn	TCAGGCTGATGCTTTTGCTCAAGAACCTTGGATATGCCCCCTGAGCTCCGAGCAGCCTTGT	860
BASF-Soy	TCAGGCTGATGCTTTTGCTCAAGAACCTTGGATATGATCTCTGATTACGCGGTGCTCTGT	1380
Consensus	TCAGGCTGATGXXTTTGCTXXXGAXCTTGGCTATGXXXXXXXTXCGXXXXXXCTXGT	1380
	1390 1400 1410 1420 1430 1440	
BASF_AT1	GAAACTACAGGAAGAGAACTTATCAGCAATGAATACTGATCCATTGTACTCAGCTTATCA	1208
BASF_AT2	GAAACTACAGGAAGAGAACTTATCAGCAATGAATACTGATCCATTGTACTCAGCTTATCA	1208
BASF-Corn	TAAACTACAGGAGGAGAACTTGTCTGCGATGAACACCGATCCTTGTATTCCGGATATCA	920
BASF-Soy	GAAACTACAGGAGGAGAACTCTGTCAGCTATGAATACAGATCC-TTGCTGTGCG-----	1434
Consensus	XAAACTACAGGAXGAGAAAXXTXCTXGCMATGAAXACXGATCXXTXGXXXXXXGXXXXXX	1440
	1450 1460 1470 1480 1490 1500	
BASF_AT1	CTACTCACATCCTCCTCTGTTGAAAGGCTTTCAGGCATTGATGAGAAGACAAAGAAGAC	1268
BASF_AT2	CTACTCACATCCTCCTCTGTTGAAAGGCTTTCAGGCATTGATGAGAAGACAAAGAAGAC	1268
BASF-Corn	CTACTCCACCCACCACTCTGAGAGGCTGCAAGCTTTGGAAGATTTCAGACGCAAAATA	980
BASF-Soy	-----	1434
Consensus	XX	1500
	1510 1520 1530 1540 1550 1560	
BASF_AT1	AGATTAA-----	1275
BASF_AT2	AGATTAA-----	1275
BASF-Corn	ACAAGATTAGTCGATCCTTGTATGAGGTTTACATATGGATTTTCCCTGCCACATGCACA	1040
BASF-Soy	-----	1434
Consensus	XX	1560

	1570	1580	1590	1600	1610	1620	
BASF_AT1	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....						1275
BASF_AT2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1275
BASF-Corn	CCGATTTCAGTGCCTGGATGGTGAGGGTTTTGACATAGGAGTGTTGTCAAAGCTTTAGAGT						1100
BASF-Soy	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1434
Consensus	XX						1620
	1630	1640	1650	1660	1670	1680	
BASF_AT1	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....						1275
BASF_AT2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1275
BASF-Corn	GCATCTTTTCGGTCAGGTGCAACAGCCTTTCGGTCATTGAGACATATAAGCGAATTAGCTA						1160
BASF-Soy	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1434
Consensus	XX						1680
	1690	1700	1710	1720	1730	1740	
BASF_AT1	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....						1275
BASF_AT2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1275
BASF-Corn	TTAAAAAAACAGAACTGTTGCATCAAAAAAAAAAAAAAAAAAGAAACAAAAAAAAAAAAA						1220
BASF-Soy	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1434
Consensus	XX						1740
	1750	1760	1770	1780	1790	1800	
BASF_AT1	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....						1275
BASF_AT2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1275
BASF-Corn	AAAAAAAAAAGAAAAAAAAAAAAAAAAAAGTGCTCTGCGTTGTTACCACTGCTTG						1280
BASF-Soy	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1434
Consensus	XX						1800
	1810	1820					
BASF_AT1	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....						1275
BASF_AT2	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1275
BASF-Corn	CCCTATAGTGATCGTATCAGA						1301
BASF-Soy	----- ----- ----- ----- ----- ----- ----- ----- ----- ----- ----- -----						1434
Consensus	XXXXXXXXXXXXXXXXXXXXXXXXXXXX						1821

Table 32. ClustalW Analysis of BASF Amino Acids

- 1) BASF\_AT1 (SEQ ID NO:117)
- 2) BASF\_AT2 (SEQ ID NO:119)
- 3) BASF-Corn (SEQ ID NO:121)
- 4) BASF-Soy (SEQ ID NO:123)
- 5) Consensus (SEQ ID NO:164)

	10	20	30	40	50	60	
BASF_AT1	MAIPEMETVVGFMIVMYIFETYLDVROLTALKLPTLPKTLVGVISOEKFEKSRAYSLDKS						60
BASF_AT2	MAIPEMETVVGFMIVMYIFETYLDVROLTALKLPTLPKTLVGVISOEKFEKSRAYSLDKS						60
BASF-Corn	MAIPEMETVVGFMIVMYIFETYLDVROLTALKLPTLPKTLVGVISOEKFEKSRAYSLDKS						1
BASF-Soy	MAIPEMETVVGFMIVMYIFETYLDVROLTALKLPTLPKTLVGVISOEKFEKSRAYSLDKS						60
Consensus BASF	XX						60
	70	80	90	100	110	120	
BASF_AT1	YFHFVHEFVTIIMDSATLFCGLPWFWMKSGAVTPRLGLEPENEILHTLSFLAGVMWWSH						120
BASF_AT2	YFHFVHEFVTIIMDSATLFCGLPWFWMKSGAVTPRLGLEPENEILHTLSFLAGVMWWSH						120
BASF-Corn	YFHFVHEFVTIIMDSATLFCGLPWFWMKSGAVTPRLGLEPENEILHTLSFLAGVMWWSH						24
BASF-Soy	YFHFVHEFVTIIMDSATLFCGLPWFWMKSGAVTPRLGLEPENEILHTLSFLAGVMWWSH						120
Consensus BASF	XX						120
	130	140	150	160	170	180	
BASF_AT1	ITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIVQKGGPY						180
BASF_AT2	ITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIVQKGGPY						180
BASF-Corn	ITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIVQKGGPY						84
BASF-Soy	ITDLPFSLYSTFVIESRHGFNKQTIWMFIRDMIKGTFLSVILGPPIVAAIIVQKGGPY						180

Consensus BASF	ITDLPPSLYSTFVIEKRRHGFNKOTXWKKRDMKGGXLSKIXGPPIVAATIKIVQXGGPY	180
	190 200 210 220 230 240	
BASF_AT1	LAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPPLKKLFVV	240
BASF_AT2	LAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPPLKKLFVV	240
BASF-Corn	LAIYLWGFMFVLAALVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPPLKKLFVV	144
BASF-Soy	LAIYLWVFTEGLSVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPPLKKLFVV	240
Consensus BASF	LAIYLWGFMFVLAALVMMTIYPVLIAPLFNKFTPLPDGDLREKIEKLASSLKFPPLKKLFVV	240
	250 260 270 280 290 300	
BASF_AT1	DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNHTIYSFIA	300
BASF_AT2	DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNHTIYSFIA	300
BASF-Corn	DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVSVIAHELGHWKLNHTIYSFIA	204
BASF-Soy	DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKDEDEIVAVIAHELGHWKLNHTIYSFIA	300
Consensus BASF	DGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKXXXNEIVSVIAHELGHWKLNHTIYXFXA	300
	310 320 330 340 350 360	
BASF_AT1	VQILAFLOFGGYTLVRNSTDLFRSFGFDTPQVLIGLIIFQHTVIPLOHLVSFGLNLVSRA	360
BASF_AT2	VQILAFLOFGGYTLVRNSTDLFRSFGFDTPQVLIGLIIFQHTVIPLOHLVSFGLNLVSRA	360
BASF-Corn	VQILAFLOFGGYTLVRSSKDLFSFGFDTPQVLIGLIIFQHTVIPLOHLVSFGLNLVSRA	264
BASF-Soy	VQILAFLOFGGYTLVRNSADLFRSFGFDTPQVLIGLIIFQHTVIPLOHLVSFGLNLVSRA	360
Consensus BASF	XQILAFLOFGGYTLVRXSKDLFSFGFDTPQVLIGLIIFQHTVIPLOHLVSFGLNLVSRA	360
	370 380 390 400 410 420	
BASF_AT1	FEFQADAFVAKLGYAKDLRPTLVKLOEENLSAMNTDPLYSAYHYSHPPPLVERLRALDGED	420
BASF_AT2	FEFQADAFVAKLGYAKDLRPTLVKLOEENLSAMNTDPLYSAYHYSHPPPLVERLRALDGED	420
BASF-Corn	FEFQADAFVAKLGYAPQLRAALVKLOEENLSAMNTDPWYSAYHYSHPPPLVERLRALDGED	324
BASF-Soy	FEFQADGFVAKLGYASGLRGGLVKLOEENLSAMNTDPCSC-----	400
Consensus BASF	FEFQADGFVAKLGYAXXLRXXLVKLOEENLSAMNTDXXXXXXXXXXXXXXXXXXXXXXXXXX	420
	430 440 450 460 470 480	
BASF_AT1	KKTD-----	424
BASF_AT2	KKTD-----	424
BASF-Corn	DKKEDSILVGLHMDFSLPHAHRFSAWMVRVLTECCQSFRVHLSVRCNSLSVIETYKRISY	384
BASF-Soy	-----	400
Consensus BASF	XX	480
	490 500 510 520 530 540	
BASF_AT1	-----	424
BASF_AT2	-----	424
BASF-Corn	KKQNCCKKKKKKETKKKKKKKKKKKKKVLVCVTTACPIVIVS-----	429
BASF-Soy	-----	400
Consensus BASF	XX	525
	550 560 570 580 590 600	
BASF_AT1	-----	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	610 620 630 640 650 660	
BASF_AT1	-----	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	670 680 690 700 710 720	
BASF_AT1	-----	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	730 740 750 760 770 780	
BASF_AT1	-----	424

BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	790 800 810 820 830 840	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	850 860 870 880 890 900	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	910 920 930 940 950 960	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	970 980 990 1000 1010 1020	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	1030 1040 1050 1060 1070 1080	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	1090 1100 1110 1120 1130 1140	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	1150 1160 1170 1180 1190 1200	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	1210 1220 1230 1240 1250 1260	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525
	1270 1280 1290 1300 1310 1320	
BASF_AT1	.... .... .... .... .... ....	424
BASF_AT2	-----	424
BASF-Corn	-----	429
BASF-Soy	-----	400
Consensus BASF	-----	525

	1330	1340	1350	1360	1370	1380
	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....					
BASF_AT1						424
BASF_AT2						424
BASF-Corn						429
BASF-Soy						400
Consensus BASF						525

Table 33. ClustalW Analysis of Generic Nucleic Acids

- 1) **afc1** (SEQ ID NO:124)
- 2) **AT4g01320** (SEQ ID NO:126)
- 3) **AF007269** (SEQ ID NO:128)
- 4) **Consensus** (SEQ ID NO:165)

	10	20	30	40	50	60
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	ATGGCGATTCTCTTCATGGAAACCGTCGTGGGTAAGCTTCAAAACCTTTTCTGAGACAT	60				
Consensus	XX	60				
	70	80	90	100	110	120
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	TTTACTATCCTGTTTCACTCATCGTATTTTCGTTTTTGGTTGGGTTTGCTTTCGTGTGTG	120				
Consensus	XX	120				
	130	140	150	160	170	180
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	TGTGTGTTGAGATTCCATGACTCGTTTGTTCATATACCATCGTCTCTGCTTCTCGTTTC	180				
Consensus	XX	180				
	190	200	210	220	230	240
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	TAAATTTTGTCTCTTTCTAATAGTGCCTACCTTGATCTGAGGTTTATTACTCCTACTAG	240				
Consensus	XX	240				
	250	260	270	280	290	300
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	TTTCTGTCTTACTCGTGCCTTGTGATTGATTGAGCTTATGTGATTTCATCATCTCTTC	300				
Consensus	XX	300				
	310	320	330	340	350	360
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	CTCGGTTTGAATGTACGGAGCTTCTCTGTTAACCAAAATCTAGGATTGGGAAGAAAA	360				
Consensus	XX	360				
	370	380	390	400	410	420
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	GTCGGAGTCTTTTTTTCCTCATTCCTGATTGGAATTGAGAACTTGAAATTTTCTTT	420				
Consensus	XX	420				
	430	440	450	460	470	480
afcl	..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... .....	1				
AT4g01320	-----	1				
AF007269	GTTCAAGTCATACAGCTTGAGGTTTGGGTTTCTGTGTCAGGTATTATTATGTTTCGTGA	480				
Consensus	XX	480				

225

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      1150      1160      1170      1180      1190      1200
afcl      GTTTACCAGAGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 336
AT4g01320 GTTTACCAGAGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 357
AF007269   GTTTACCAGAGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 1200
Consensus  GTTTACCAGAGTTGGGCCTTGATCCAGAGAATGAAATACTGCATACTCTTTCATTCTTG 1200

      1210      1220      1230      1240      1250      1260
afcl      GCTGGTGTATGACATGGTCACAG----- 360
AT4g01320 GCTGGTGTATGACATGGTCACAG----- 381
AF007269   GCTGGTGTATGACATGGTCACAGGTGTCCAAATAAACCCCTTCATATAGTCTTATACG 1260
Consensus  GCTGGTGTATGACATGGTCACAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1260

      1270      1280      1290      1300      1310      1320
afcl      ----- 360
AT4g01320 ----- 381
AF007269   TTTAGCATCAAAATATCTATTTCTTAAGATAATAATATTCTTTATATCTGATGCAG 1320
Consensus  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1320

      1330      1340      1350      1360      1370      1380
afcl      ATCACTGATTGCCATTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTG 420
AT4g01320 ATCACTGATTGCCATTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTG 441
AF007269   ATCACTGATTGCCATTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTG 1380
Consensus  ATCACTGATTGCCATTTCTTTGTACTCAACTTTCGTGATCGAGTCTCGGCATGGGTTG 1380

      1390      1400      1410      1420      1430      1440
afcl      AACAAA----- 426
AT4g01320 AACAAA----- 447
AF007269   AACAAAGTATGTCGTATTTCCAACACTACCTTGTGACTTACGTTTTTTATCAGAGATGT 1440
Consensus  AACAAAXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1440

      1450      1460      1470      1480      1490      1500
afcl      -----CAAACAATATGGATGTTTCATTAGGGACA 454
AT4g01320 -----CAAACAATATGGATGTTTCATTAGGGACA 475
AF007269   GGATTAAATTGCTTCTAAATTCTGTTGACACCAAACAATATGGATGTTTCATTAGGGACA 1500
Consensus  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXCAAACAATATGGATGTTTCATTAGGGACA 1500

      1510      1520      1530      1540      1550      1560
afcl      TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT 514
AT4g01320 TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT 535
AF007269   TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT 1560
Consensus  TGATCAAAGGAACATTCTCTCTGTCTACTAGGCCACCCATTGTTGCTGCGATAATTT 1560

      1570      1580      1590      1600      1610      1620
afcl      TCATAGTCCAG----- 525
AT4g01320 TCATAGTCCAG----- 546
AF007269   TCATAGTCCAGTTTGATGATTCTGATTCTCTTATTTCTGAGTTTTTCACATGGATGA 1620
Consensus  TCATAGTCCAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1620

      1630      1640      1650      1660      1670      1680
afcl      ----- 525
AT4g01320 ----- 546
AF007269   CTATTCTCCATTGAGTGTGAGCTTCAAAGTTTTAGTTTTCTGTTAAAAATTTAAATTT 1680
Consensus  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1680

      1690      1700      1710      1720      1730      1740
afcl      -----AAAGGAGGTCCTTATCTTGCCATC 549
AT4g01320 -----AAAGGAGGTCCTTATCTTGCCATC 570
AF007269   TGCTTCTCTGAGCATGAAGTTTCTATCTTTTCCAGAAAGGAGGTCCTTATCTTGCCATC 1740
Consensus  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXAAAGGAGGTCCTTATCTTGCCATC 1740

      1750      1760      1770      1780      1790      1800
afcl      TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 609
AT4g01320 TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 630
AF007269   TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 1800
Consensus  TATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGACTATATACCCGGTCTTG 1800

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      1810      1820      1830      1840      1850      1860
afcl      ATAGCACCCTCTTCAACAAGTTCACTCCT----- 639
AT4g01320 ATAGCACCCTCTTCAACAAGTTCACTCCT----- 660
AF007269  ATAGCACCCTCTTCAACAAGTTCACTCCTGTGTGATTTCTGTCATGGCCATTTTACAA 1860
Consensus ATAGCACCCTCTTCAACAAGTTCACTCCTXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1860

      1870      1880      1890      1900      1910      1920
afcl      ----- 639
AT4g01320 ----- 660
AF007269  TTCACTGCTTGTGTCATATGTTGTACCAGACAATATAATCTCCCGCTTTTATGGCT 1920
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 1920

      1930      1940      1950      1960      1970      1980
afcl      CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT 695
AT4g01320 CTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT 716
AF007269  ATAGCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT 1980
Consensus XXXCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTCTCTAAAGTT 1980

      1990      2000      2010      2020      2030      2040
afcl      TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG 751
AT4g01320 TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG 772
AF007269  TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAG 2040
Consensus TCCTTTGAAGAAGCTGTTTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGXXXX 2040

      2050      2060      2070      2080      2090      2100
afcl      ----- 751
AT4g01320 ----- 772
AF007269  AAGCTTGAGATCTCTTCTACCTACTTACTCTAGTTTACCATTAGAAGCTTACGTATCT 2100
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2100

      2110      2120      2130      2140      2150      2160
afcl      CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 795
AT4g01320 CTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 816
AF007269  TGTACATCATACAGGCTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 2160
Consensus XXXXXXXXXXXXXXXCTTACATGTATGGTTTCTTTAAGAACAAAAGGATTGTTCTTTAT 2160

      2170      2180      2190      2200      2210      2220
afcl      GATACGTTGATTGAGCAG----- 813
AT4g01320 GATACGTTGATTGAGCAG----- 834
AF007269  GATACGTTGATTGAGCAGGTACTGTGACTCTTGATGCTTCAACGAGCTATACTCACATT 2220
Consensus GATACGTTGATTGAGCAGXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2220

      2230      2240      2250      2260      2270      2280
afcl      ----- 829
AT4g01320 ----- 850
AF007269  TCTGTTTCTGGTTCTGAAACATAACATAATCTTCTATTGTGCAGTGAAGAATGAGGATG 2280
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXTGAAGAATGAGGATG 2280

      2290      2300      2310      2320      2330      2340
afcl      AAATGTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT 889
AT4g01320 AAATGTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT 910
AF007269  AAATGTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT 2340
Consensus AAATGTGTGGCGGTTATTGCACACGAGCTTGGACATTGGAACTGAATCACACTACATACT 2340

      2350      2360      2370      2380      2390      2400
afcl      CGTTCAATTGCAGTTCAA----- 906
AT4g01320 CGTTCAATTGCAGTTCAA----- 927
AF007269  CGTTCAATTGCAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACCTACTCACATCTACAT 2400
Consensus CGTTCAATTGCAGTTCAAXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 2400

      2410      2420      2430      2440      2450      2460
afcl      ----- 915
AT4g01320 ----- 936
AF007269  TTCACTTAAGAAATCATGTCTTATGACCCTCTCTCAATGTTTGTGCTGCAGATCCTTGCC 2460
Consensus XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXATCCTTGCC 2460

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	2470	2480	2490	2500	2510	2520	
afcl	TTCTTACAATTTGGAGGATACACTCTTGT	CAGAACTCCACTGATCTCTTCAGGAGTTTC	975				
AT4g01320	TTCTTACAATTTGGAGGATACACTCTTGT	CAGAACTCCACTGATCTCTTCAGGAGTTTC	996				
AF007269	TTCTTACAATTTGGAGGATACACTCTTGT	CAGAACTCCACTGATCTCTTCAGGAGTTTC	2520				
Consensus	TTCTTACAATTTGGAGGATACACTCTTGT	CAGAACTCCACTGATCTCTTCAGGAGTTTC	2520				
	2530	2540	2550	2560	2570	2580	
afcl	GGATTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTTCAG	-----	1020				
AT4g01320	GGATTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTTCAG	-----	1041				
AF007269	GGATTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTTCAG	TTTGTATTATTTTTC	2580				
Consensus	GGATTGATACACAGCCTGTTCTCATTGGTTTGATCATATTTTCAG	XXXXXXXXXXXXXXXXXX	2580				
	2590	2600	2610	2620	2630	2640	
afcl	-----	-----	-----	-----	-----	-----	1020
AT4g01320	-----	-----	-----	-----	-----	-----	1041
AF007269	CTTTTGACACTAATCTAATGAATCAAGGATGGATTAAGAAAAAACTCTAAACCTTTG	-----	2640				
Consensus	XX	-----	2640				
	2650	2660	2670	2680	2690	2700	
afcl	-----	-----	CACACTGTAATACCACTGCAACATCTAGTAAGC	-----	-----	-----	1053
AT4g01320	-----	-----	CACACTGTAATACCACTGCAACATCTAGTAAGC	-----	-----	-----	1074
AF007269	GTTATATCTCCTGTCTGATTATCACAG	CACACTGTAATACCACTGCAACATCTAGTAAGC	-----	-----	-----	-----	2700
Consensus	XXXXXXXXXXXXXXXXXXXXXXXXXXXX	CACACTGTAATACCACTGCAACATCTAGTAAGC	-----	-----	-----	-----	2700
	2710	2720	2730	2740	2750	2760	
afcl	TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG	-----	-----	-----	-----	-----	1093
AT4g01320	TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG	-----	-----	-----	-----	-----	1114
AF007269	TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG	TACCATCTTACAATCCCTCA	-----	-----	-----	-----	2760
Consensus	TTTGGCCTGAACCTCGTTAGTCGAGCGTTTGAGTTTCAGG	XXXXXXXXXXXXXXXXXXXX	-----	-----	-----	-----	2760
	2770	2780	2790	2800	2810	2820	
afcl	-----	-----	-----	-----	-----	-----	1093
AT4g01320	-----	-----	-----	-----	-----	-----	1114
AF007269	AGATCCAACCATAGTTTCTTTATTGCAATGCGAGCCTCATCTACTAATCTGAGTTAACGT	-----	-----	-----	-----	-----	2820
Consensus	XX	-----	-----	-----	-----	-----	2820
	2830	2840	2850	2860	2870	2880	
afcl	-----	CTGATGCTTTTGC	GTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG	-----	-----	-----	1141
AT4g01320	-----	CTGATGCTTTTGC	GTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG	-----	-----	-----	1162
AF007269	TCCTTTTGCAAGCTGATGCTTTTGC	GTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG	-----	-----	-----	-----	2880
Consensus	XXXXXXXXXXXX	CTGATGCTTTTGC	GTGAAGCTTGGCTATGCAAAAGATCTTCGTCCTG	-----	-----	-----	2880
	2890	2900	2910	2920	2930	2940	
afcl	CTCTAGTGAAACTACAGG	-----	-----	-----	-----	-----	1159
AT4g01320	CTCTAGTGAAACTACAGG	CTCAGAGAAGATAACAACAGAACACAACTGTTACCTCAATTT	-----	-----	-----	-----	1222
AF007269	CTCTAGTGAAACTACAGG	CTCAGAGAAGATAACAACAGAACACAACTGTTACCTCAATTT	-----	-----	-----	-----	2940
Consensus	CTCTAGTGAAACTACAGG	XX	-----	-----	-----	-----	2940
	2950	2960	2970	2980	2990	3000	
afcl	-----	-----	-----	-----	-----	-----	1177
AT4g01320	GTGTCACACACTTAAATGGATT	TTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA	-----	-----	-----	-----	1282
AF007269	GTGTCACACACTTAAATGGATT	TTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA	-----	-----	-----	-----	3000
Consensus	XXXXXXXXXXXXXXXXXXXXXXXXXXXX	TTTTGTTGGGATTTTGCAGGAAGAGAACTTATCAGCAA	-----	-----	-----	-----	3000
	3010	3020	3030	3040	3050	3060	
afcl	TGAACACTGATCCATTG	CACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC	-----	-----	-----	-----	1237
AT4g01320	TGAACACTGATCCATTG	CACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC	-----	-----	-----	-----	1342
AF007269	TGAACACTGATCCATTG	CACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC	-----	-----	-----	-----	3060
Consensus	TGAACACTGATCCATTG	CACTCAGCTTATCACTACTCACATCCTCCTCTTGTGAAAGGC	-----	-----	-----	-----	3060
	3070	3080	3090				
afcl	TTTCAGGCCATTGATGGAGAAGACAAGAAGACAGATTAA	-----	-----	1275			
AT4g01320	TTTCAGGCCATTGATGGAGAAGACAAGAAGACAGATTAA	-----	-----	1380			
AF007269	TTTCAGGCCATTGATGGAGAAGACAAGAAGACAGATTAA	-----	-----	3098			
Consensus	TTTCAGGCCATTGATGGAGAAGACAAGAAGACAGATTAA	-----	-----	3098			

**Table 34. ClustalW Analysis of Generic Amino Acids**

1) **afc1** (SEQ ID NO:125)  
2) **AT4g01320** (SEQ ID NO:127)  
3) **AF007269** (SEQ ID NO:129)  
4) **Consensus** (SEQ ID NO:166)

**a**

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      10          20          30          40          50          60
afcl       MAIPFMETVVGFIMVYIFETYLDLRQLTALKLPTLPKTLVGVISQEFKEFSRAYS--DD 58
AT4g01320 MAIPFMETVVGFIMVYIFETYLDLRQLTALKLPTLPKTLVGVISQEFKEFSRAYRDIT 60
AF007269   MAIPFMETVVGFIMVYIFETYLDLRQLTALKLPTLPKTL----- 41
Consensus Publi MAIPFMETVVGFIMVYIFETYLDLRQLTALKLPTLPKTLXXXXXXXXXXXXXXXXXXXXX 60

      70          80          90          100         110         120
afcl       ---SYFHFVHFEFVTILMDSAILFFGILPWFWKMMSGAVLPRGLDLPENEILHTLSFLA 113
AT4g01320 ENFNICSYFHFVHFEFVTILMDSAILFFGILPWFWKMMSGAVLPRGLDLPENEILHTLSFLA 120
AF007269   -----T----- 42
Consensus Publi XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 120

      130         140         150         160         170         180
afcl       GVMTWSQLTDLPFSLYSTFVIESRHGFNKTIIWMFIRDMIKGTFLSVLGGPPIVAAIIFI 173
AT4g01320 GVMTWSQLTDLPFSLYSTFVIESRHGFNKTIIWMFIRDMIKGTFLSVLGGPPIVAAIIFI 180
AF007269   --DLPFSLYSTFVIESRHGFNKTIIWMFIRDMIKGTFLSVLGGPPIVAAIIFI 93
Consensus Publi XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 180

      190         200         210         220         230         240
afcl       VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLEKIEKLASSLKFP 233
AT4g01320 VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLEKIEKLASSLKFP 240
AF007269   VQKGGPYLAIYLWAFMFILSLVMMTIYPVLIAPLFNKFTPLPDGDLEKIEKLASSLKFP 153
Consensus Publi XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 240

      250         260         270         280         290         300
afcl       LKKLVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH 293
AT4g01320 LKKLVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH 300
AF007269   LKKLVVDGSTRSSHSNAYMYGFFKNKRIVLYDTLIQQCKNEDEIVAVIAHELGHWKLNH 213
Consensus Publi XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 300

      310         320         330         340         350         360
afcl       TTYSFIAVQILAFLQPGGYTLVRNSTDLFRSFGFDTPQVLIGLIIFOHTVIPLQHLVSFG 353
AT4g01320 TTYSFIAVQILAFLQPGGYTLVRNSTDLFRSFGFDTPQVLIGLIIFOHTVIPLQHLVSFG 360
AF007269   TTYSFIAV-----QHTVIPLOHLVSFG 235
Consensus Publi XXXXXXXXXXXXXXXXXXXXXXXXXX|XXXXXXXXXXXXXXXXXXXXXXXXXXXX|XXQHXVXXXXXLVSXX 360

      370         380         390         400         410         420
afcl       LNLVSRAFEFAQDAFAVKLGyakDLRPALVKLQ----- 386
AT4g01320 LNLVSRAFEFAQDAFAVKLGyakDLRPALVKLQVREDNNRTQTITSICVTHLNGFFVGIL 420
AF007269   LNLVSRAFEFAQDAFAVKLGyakDLRPALVKLQVREDNNRTQT----- 278
Consensus Publi XXXXXXXXAFXXXXXXXXXXXXXXXXXX|XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXGXX 420

      430         440         450         460         470         480
afcl       -EENLSAMNTDPLISAYHYSHPPVERLRAIDGEDDKKD- 424
AT4g01320 QEENLSAMNTDPLYSAYHYSHPPVERLRAIDGEDDKKD- 459
AF007269   -EENLSAMNTDPLYSAYHYSHPPVERLRAIDGEDDKKD- 316
Consensus Publi XXXXXLSAMNTDPLYSAYHYSHPPVERLRAIDGEDDKKD- 480
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**Table 35. ClustalW Analysis of PPI Nucleic Acids**

1) PPI-AtCPP (SEQ ID NO:97)

- 2) PPI-BnCPP (SEQ ID NO:109)  
 3) PPI-SoyCPP (SEQ ID NO:112)  
 4) Consensus (SEQ ID NO:167)

	10	20	30	40	50	60
PPI-AtCPP	ATGGCGATTCC	TTTCATGGA	AACCGTCG	TGGTTTATG	ATAGTGAT	TACATTTT
PPI-BnCPP	ATGGCGATTCC	TTTCATGGA	AACCGTCG	TGGTTTATG	ATAGTGAT	TACATTTT
PPI-SoyCPP	ATGGCGTTCC	CTACATGGA	ACCGTTGT	CGGATTTAT	GATATTAAT	GTACATTTT
Consensus	ATGGCGTTCC	CTACATGGA	ACCGTTGT	CGGATTTAT	GATATTAAT	GTACATTTT
	70	80	90	100	110	120
PPI-AtCPP	ACGTATTTGG	ATCTGAGG	CAACTCACT	GCTCTCAAG	CTTCCAACT	CTCCCCGAA
PPI-BnCPP	ACGTATTTGG	ATCTGAGG	CAACTCACT	GCTCTCAAG	CTTCCAACT	CTCCCCGAA
PPI-SoyCPP	ACTTACTTGG	ATCTGAG	CAACATAG	GCGCCCTCA	AACTTCCCT	ACTCTTCCA
Consensus	ACXTATTTGG	ATCTGAG	CAACATAG	GCGCCCTCA	AACTTCCCT	ACTCTTCCA
	130	140	150	160	170	180
PPI-AtCPP	GTTGGTGTA	AATTAGCC	AAGAGAAG	TTTGAGAA	ATCAGAGCA	TACAGTCTT
PPI-BnCPP	GTTGGTGTA	AATTAGCC	AAGAGAAG	TTTGAGAA	ATCAGAGCA	TACAGTCTT
PPI-SoyCPP	GAGGCTGT	TATCAGCC	AAGAGAAT	TTGAGAA	ATCTAGAGC	CTATAGTCTT
Consensus	GXXGCTGT	TATCAGCC	AAGAGAAT	TTGAGAA	ATCTAGAGC	CTATAGTCTT
	190	200	210	220	230	240
PPI-AtCPP	TATTTTCACT	TTTGTTCAT	GAGTTTGT	TAATACTAT	ACTTATGGA	CTGCAATTT
PPI-BnCPP	CATTTTCACT	TTTGTTCAT	GAGTTTGT	TAATACTAT	ACTTATGGA	CTGCAATTT
PPI-SoyCPP	CACTTTCCAT	TTTGTTC	AGTTTGTG	ACAAATAGT	GACAGACTC	TACAATTTT
Consensus	XAXTTTCACT	TTTGTTC	AGTTTGTG	ACAAATAGT	GACAGACTC	TACAATTTT
	250	260	270	280	290	300
PPI-AtCPP	GGGATCTTG	CCCTGGT	TTTGGAA	GATCTCTGG	AGCTCTT	TACCCAGCT
PPI-BnCPP	GGGATCTTG	CCCTGGT	TTTGGAA	GATCTCTGG	AGCTCTT	TACCCAGCT
PPI-SoyCPP	GGGCTATTG	CCCTGGT	TTTGGAA	GAATCAGG	AGATTTTAT	GACAATAGT
Consensus	GGGCTATTG	CCCTGGT	TTTGGAA	GAATCAGG	AGATTTTAT	GACAATAGT
	310	320	330	340	350	360
PPI-AtCPP	CCGAGAAAT	GAAATACT	GCATACT	CTTTCATT	CTCTGGCT	GGTCTTATG
PPI-BnCPP	CCGAGAAAT	GAAATACT	GCATACT	CTTTCATT	CTCTGGCT	GGTCTTATG
PPI-SoyCPP	GCTGAGAAT	GAAATACT	GCATACT	CTTTCATT	CTCTGGCT	GGTCTTATG
Consensus	XCTGAGAAT	GAAATACT	GCATACT	CTTTCATT	CTCTGGCT	GGTCTTATG
	370	380	390	400	410	420
PPI-AtCPP	ATCACTGAT	TTTGCCAT	TTTCTTT	GTACTCA	ACTTTT	CGTGATCG
PPI-BnCPP	ATCACTGAT	TTTGCCAT	TTTCTTT	GTACTCA	ACTTTT	CGTGATCG
PPI-SoyCPP	ATAACAGAT	TTTGCC	TTTTCT	GTACTCA	ACTTTT	GTGATTG
Consensus	ATXAGGAT	TTTGCC	TTTTCT	GTACTCA	ACTTTT	GTGATTG
	430	440	450	460	470	480
PPI-AtCPP	AACAACA	AAATATG	GATGTT	CATTAGG	GACATG	ATCAAGGA
PPI-BnCPP	AACAACA	AAATATG	GATGTT	CATTAGG	GACATG	ATCAAGGA
PPI-SoyCPP	AATAAC	CAACA	CCATGG	TATTCT	TTAGG	GACATG
Consensus	AAXAACA	CAACA	CCATGG	TATTCT	TTAGG	GACATG
	490	500	510	520	530	540
PPI-AtCPP	ATACTAGG	CCACCC	ATTGTT	GCTGC	GATAATTT	TATAGTCC
PPI-BnCPP	ATAGCTGC	CCCTCCT	ATCGTT	GCGCAAT	TATGTT	TATAGTCC
PPI-SoyCPP	ATAATGG	CCACCT	ATTGTT	GCTGC	CAATCATT	GTAATAGT
Consensus	ATAXXG	CCXCC	ATTGTT	GCTGC	CAATCATT	GTAATAGT
	550	560	570	580	590	600
PPI-AtCPP	CTTGCCAT	CTATCTG	TGGGCAT	TTCATG	TTTATCCT	GTCTCTAG
PPI-BnCPP	CTTGCCAT	CTATCTG	TGGGCAT	TTCATG	TTTATCCT	GTCTCTAG
PPI-SoyCPP	TTGGCCAT	CTATCT	TGGGTTT	TACGTTT	GGTCTT	CTATTG
Consensus	XTTGCCAT	CTATCT	TGGGTTT	TACGTTT	GGTCTT	CTATTG
	610	620	630	640	650	660
	.....	.....	.....	.....	.....	.....

PPI-AtCPP	CCGTCCTTGATAGCACCGCTCTTCAACAAATTCACCTCCTCTCCAGATGGAGACCTCCGG	660
PPI-BnCPP	CCTGTTTGTATGACCTCTTTCAACAAGTTCACTCCTCTTCCATGGAGACCTCCGG	660
PPI-SoyCPP	CCAGTACTAATAGCTCCACTCTTCAATAAGTTCACTCCACTTCCAGATGCTCACTCAGG	660
Consensus	CCXGTXXTKATXGXXCCXCTXTCAAAAXTTCACTCCXCTTCCATGGAGXXAXCTCXXG	660
	670 680 690 700 710 720	
PPI-AtCPP	GAGAAGATTGAGAACTTGCTTCTTCCCTAAAGTTTCCTTTGAAGAAGCTGTTTGTGTGTC	720
PPI-BnCPP	GAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCCTTTGAAGAAGCTGTTTGTGTGTC	720
PPI-SoyCPP	GAGAAATCGAGAACTTGCTTCTTCCCTCAACTATCCGTTAAAGAACTATTGTGTGTC	720
Consensus	GAGAAMATXGAGAACTTGCTTCTTCTCTAAAXTTCCTTAAAGAACTXTTTGTGTGTC	720
	730 740 750 760 770 780	
PPI-AtCPP	GATGGATCTACAAGGTCAAGCCATAGCAATGCTTACATGTATGGTTTCTTTAAGAACAAA	780
PPI-BnCPP	GATGGATCTACAAGGTCAAGCCATAGTAATGCTTACATGTATGGTTTCTTCAAGAACAAA	780
PPI-SoyCPP	GATGGATCTACAAGTCAAGTCAAGCAATGCTATGTATGGTTTCTTCAAGAACAAG	780
Consensus	GATGGATCTACAAGTCAAGXCAXAGTAATGGXTATATGTATGGTTTCTTAAAGAACAAX	780
	790 800 810 820 830 840	
PPI-AtCPP	AGGATTGTTCTTTATGATACGTTGATTGAGCAGTGCAGAAATGAGGATGAAATTTGTGGCG	840
PPI-BnCPP	AGGATTGTTCTTTATGACACATTGATTGAGCAGTGCAGAAATGAGGATGAAATTTGTGGCG	840
PPI-SoyCPP	AGGATTGTTCTTTATGACACATTATTTCAACAGTGCAGAAATGAGGATGAAATTTGTGGCG	840
Consensus	AGGATTGTTCTTTATGAXCTTATTCAXCAGTGCAXXAXGAXXAXGAAATTTGTGGCG	840
	850 860 870 880 890 900	
PPI-AtCPP	GTTATTGACACAGAGCTTGGACATTGGAACTGAATCACACTACATACCTCGTTTATTGGA	900
PPI-BnCPP	GTTATTGACACAGAGCTGGGACACTGGAAGCTGAATCACACTACATACCTCGTTTATTGCT	900
PPI-SoyCPP	GTTATTGACCATGAGTTGGGACACTGGAAGCTCAACCATCTGTGTACACATTGTTGCT	900
Consensus	GTTATTGCTCAXGAGXTXGGACATGGAACTXAAACAXACTXXXTACXCTTXXTTGGX	900
	910 920 930 940 950 960	
PPI-AtCPP	GTTCAAATCCTTGCTTCTTACAATTTGGAGGATACACTCTTCTCAGAACTCCACTGAT	960
PPI-BnCPP	GTTCAAATCCTTGCTTCTTGAATTTGGAGGATACACTCTTGTGAGAACTCCACTGAT	960
PPI-SoyCPP	ATGCACATTCTTACACTTCTACAATTTGGAGGATATACACTAGTCCGAAATTCAGCTGAT	960
Consensus	XTXCAATXCTTXXXTXXTCAATTTGGAGGATAXACXCTXXTXXGAAATXXCTGAT	960
	970 980 990 1000 1010 1020	
PPI-AtCPP	CTCTTCAGGAGTTTGGATTGATACAGCCTGTTCTCATTGGTTTGATCATATTTTCA	1020
PPI-BnCPP	CTCTTCAGGAGTTTGGTTTGTATACACACCAGTTCTCATTGGTTTGATCATATTTTCA	1020
PPI-SoyCPP	CTGTATCGAAGCTTTGGTTTGTATACAGCCAGTCTCATTGGGCTCATCATATTTTCA	1020
Consensus	CTXTXXXGAGXTTXXGXTTGTATACXACXGXTCTCATTGGXXTATCATATTTTCA	1020
	1030 1040 1050 1060 1070 1080	
PPI-AtCPP	CACACTGTAATACCACTGCAACATCTAGTAAGCTTTGGCCTGAACCTCCTTAGTCGAGCG	1080
PPI-BnCPP	CACACTGTAATACCACTTCAACACCTAGTAAGCTTTGACCTCAACCTTGTAGTCGAGCG	1080
PPI-SoyCPP	CATACGTGAATCCCACTTCAGCAATTGTCAGCTTTGGTCTGAACCTAGTCAGCCGATGA	1080
Consensus	CAXACTGTAATXCCACTXCAXCAXXTXTAGCTTTGXXCTXAACTXTXAGXCGAXGX	1080
	1090 1100 1110 1120 1130 1140	
PPI-AtCPP	TTTGAGTTTCAGGCTGATGCTTTTGCTGTGAAGCTTGACTATGCAAAAATCTTCGTCCT	1140
PPI-BnCPP	TTTGAGTTTCAGGCTGATGCTTTTGCAAGTGAATCTTGGTTATGCAAAAGATCTACGTCCT	1140
PPI-SoyCPP	TTTGAAATTCAGGCTGATGGCTTTGCAAGAAGCTTGGATATGCATCTGATTACGCGGT	1140
Consensus	TTTGATTTTCAGGCTGATGXTTTGXXXXGAXCTTGGXTATGCAXXXGXXXTXCGXXX	1140
	1150 1160 1170 1180 1190 1200	
PPI-AtCPP	GCTCTAGTGAACTACAGGAAGAGAACTTATCAACAATGAACACTGATCCATTGTACTCA	1200
PPI-BnCPP	GCTCTAGTGAACTACAGGAAGAGAACTTATCAGCGATGAACACAGACCCATTGTACTCA	1200
PPI-SoyCPP	GGTCTTGTGAACTACAGGAGGAGAACTGTGCAGCTATGAATACAGATCCTTGTACTCT	1200
Consensus	GXXCTXGTGAAXCTACAGGAXGAGAAXTTCAAXCATGAAXACXGAXCGXTXGTACTGX	1200
	1210 1220 1230 1240 1250 1260	
PPI-AtCPP	GCTTATCACTACTCACATCCTCCTCTTGTGAAAGGCTTCGAGGCCATGATGGAGAAGAC	1260
PPI-BnCPP	GCTTATCACTACTCACACCTCCTCTTGTGAGAGGCTTCGAGGCCATTGATGGAGAAGAC	1260
PPI-SoyCPP	GCTTATCACTATTCTCATCTCCCTTGTGTAAGATTGGCCGCTGAGCAACCCGAT	1260
Consensus	GCTTATCACTAXTCXACCTCCCTTGTGAXAGXXTXXXXGXXXXGAXGAXXXGAX	1260
	1270	
	.... .... ....	

PPI-AtCPP AAGAAGACAGATTAA 1275  
PPI-BnCPP AAGAAGACAGATTAA 1275  
PPI-SoyCPP AAGAAGACAGATTAA 1275  
Consensus AAGAAGACAGATTAA 1275

Table 36. ClustalW Analysis of PPI Amino Acids

- 1) PPI-AtCPP (SEQ ID NO:98)
- 2) PPI-BnCPP (SEQ ID NO:110)
- 3) PPI-SoyCPP (SEQ ID NO:113)
- 4) Consensus (SEQ ID NO:168)

	10	20	30	40	50	60		
PPI-AtCPP	MAFPYMEAVVGFMI	LMYIFETYLDVROH	RALKLPTLPKTL	EGVISQEKFEKSR	AYS	LDKS	60	
PPI-BnCPP	MAFPYMEAVVGFMI	LMYIFETYLDVROH	RALKLPTLPKTL	EGVISQEKFEKSR	AYS	LDKS	60	
PPI-SoyCPP	MAFPYMEAVVGFMI	LMYIFETYLDVROH	RALKLPTLPKTL	EGVISQEKFEKSR	AYS	LDKS	60	
Consensus PPI	MAFPYMEAVVGFMI	LMYIFETYLDVROH	RALKLPTLPKTL	EGVISQEKFEKSR	AYS	LDKS	60	
	70	80	90	100	110	120		
PPI-AtCPP	HFHFVHEFVTITD	STILYFGVLPWF	WKSGDFMTIAG	FNAENILHTLA	FLAGLMIWSQ		120	
PPI-BnCPP	HFHFVHEFVTITD	STILYFGVLPWF	WKSGDFMTIAG	FNAENILHTLA	FLAGLMIWSQ		120	
PPI-SoyCPP	HFHFVHEFVTITD	STILYFGVLPWF	WKSGDFMTIAG	FNAENILHTLA	FLAGLMIWSQ		120	
Consensus PPI	HFHFVHEFVTITD	STILYFGVLPWF	WKSGDFMTIAG	FNAENILHTLA	FLAGLMIWSQ		120	
	130	140	150	160	170	180		
PPI-AtCPP	ITDLPFSLYSTF	VI EARHG FNKQ	T P W L F F R D	M L K G I F L S	V I G P P I V A A I	I V I V Q K G G P Y	180	
PPI-BnCPP	ITDLPFSLYSTF	VI EARHG FNKQ	T P W L F F R D	M L K G I F L S	V I G P P I V A A I	I V I V Q K G G P Y	180	
PPI-SoyCPP	ITDLPFSLYSTF	VI EARHG FNKQ	T P W L F F R D	M L K G I F L S	V I G P P I V A A I	I V I V Q K G G P Y	180	
Consensus PPI	ITDLPFSLYSTF	VI EARHG FNKQ	T P W L F F R D	M L K G I F L S	V I G P P I V A A I	I V I V Q K G G P Y	180	
	190	200	210	220	230	240		
PPI-AtCPP	LAIYLWVETFG	LSIVMMTLYP	VLIAPLFNKFT	PLPDGOLREK	IEKLASSLNY	PLKKLFVV	240	
PPI-BnCPP	LAIYLWVETFG	LSIVMMTLYP	VLIAPLFNKFT	PLPDGOLREK	IEKLASSLNY	PLKKLFVV	240	
PPI-SoyCPP	LAIYLWVETFG	LSIVMMTLYP	VLIAPLFNKFT	PLPDGOLREK	IEKLASSLNY	PLKKLFVV	240	
Consensus PPI	LAIYLWVETFG	LSIVMMTLYP	VLIAPLFNKFT	PLPDGOLREK	IEKLASSLNY	PLKKLFVV	240	
	250	260	270	280	290	300		
PPI-AtCPP	DGSTRSSHNSA	MYGFFKNKRIV	YDTL IQQC	KDDEIVAVIA	HELGHWKLNHT	VYTFVA	300	
PPI-BnCPP	DGSTRSSHNSA	MYGFFKNKRIV	YDTL IQQC	KDDEIVAVIA	HELGHWKLNHT	VYTFVA	300	
PPI-SoyCPP	DGSTRSSHNSA	MYGFFKNKRIV	YDTL IQQC	KDDEIVAVIA	HELGHWKLNHT	VYTFVA	300	
Consensus PPI	DGSTRSSHNSA	MYGFFKNKRIV	YDTL IQQC	KDDEIVAVIA	HELGHWKLNHT	VYTFVA	300	
	310	320	330	340	350	360		
PPI-AtCPP	MQIILTLQFG	GYTLVRNSAD	LYRSFGFD	TPVLIGLII	FQHTVPI	PLQQLVSFGLN	LVSRS	360
PPI-BnCPP	MQIILTLQFG	GYTLVRNSAD	LYRSFGFD	TPVLIGLII	FQHTVPI	PLQQLVSFGLN	LVSRS	360
PPI-SoyCPP	MQIILTLQFG	GYTLVRNSAD	LYRSFGFD	TPVLIGLII	FQHTVPI	PLQQLVSFGLN	LVSRS	360
Consensus PPI	MQIILTLQFG	GYTLVRNSAD	LYRSFGFD	TPVLIGLII	FQHTVPI	PLQQLVSFGLN	LVSRS	360
	370	380	390	400	410	420		
PPI-AtCPP	FEFQADGFAK	KLGYASGLR	GGLVQLQEEN	LSAMNTDP	WYSAYHYSH	PPPLVERLAAL	DEPD	420
PPI-BnCPP	FEFQADGFAK	KLGYASGLR	GGLVQLQEEN	LSAMNTDP	WYSAYHYSH	PPPLVERLAAL	DEPD	420
PPI-SoyCPP	FEFQADGFAK	KLGYASGLR	GGLVQLQEEN	LSAMNTDP	WYSAYHYSH	PPPLVERLAAL	DEPD	420
Consensus PPI	FEFQADGFAK	KLGYASGLR	GGLVQLQEEN	LSAMNTDP	WYSAYHYSH	PPPLVERLAAL	DEPD	420
	430	440	450	460	470	480		
PPI-AtCPP	KKED						424	
PPI-BnCPP	KKED						424	
PPI-SoyCPP	KKED						424	
Consensus PPI	KKED						480	

**Table 37. ClustalW Analysis of PPI/Generic Nucleic Acids**

- 1) **PPI-AtCPP** (SEQ ID NO:97)
- 2) **PPI-BnCPP** (SEQ ID NO:109)
- 3) **PPI-SoyCPP** (SEQ ID NO:112)
- 4) **afc1** (SEQ ID NO:124)
- 5) **AT4g01320** (SEQ ID NO:126)
- 6) **AF007269** (SEQ ID NO:128)
- 6) **Consensus** (SEQ ID NO:170)

	10	20	30	40	50	60	70
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	-----						
PPI-SoyCPP	-----						
afc1	-----						
AT4g01320	-----						
AF007269	ATGGCGATTCCCTTCATGGAAACCGTCGTGGGTAAGCTTCAAAACCTTTTCTGAGACATTTACTATCC						
Consensus	-----						
	80	90	100	110	120	130	140
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	-----ATGGCGTTTCCCTACATGGAAGCCGTTGTCGGATTATGATATTAATGTACATTTTGGAA						
PPI-SoyCPP	-----ATGGCGTTTCCCTACATGGAAGCCGTTGTCGGATTATGATATTAATGTACATTTTGGAA						
afc1	-----						
AT4g01320	-----						
AF007269	TGTTTCACTCATCGTATTTCTGTTTTGTTGGGTTTGTCTTCTGTGTGTGTGTGTGAGATTCCATGA						
Consensus	-----ATGGCGATTCCCTTCATGGAAACCGTCGT-GGTTTTATGATAT--ATGTACATTTTGGAA						
	150	160	170	180	190	200	210
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	ACTTACTTGGATG-TGCGACAACATAGGGCCCTCAAACCTCCTACTCTTCCAAAGACTTTAGAGGGTGT						
PPI-SoyCPP	ACTTACTTGGATG-TGCGACAACATAGGGCCCTCAAACCTCCTACTCTTCCAAAGACTTTAGAGGGTGT						
afc1	-----						
AT4g01320	-----						
AF007269	-CTCGTTTGTTCATATACCATCGTCTCTGCTTCTCGTTTCTAAATTTGTCTTTCTAATAGTGCCTGA						
Consensus	--CTATTGGAT---TGGCAACATG---CCTCAA--CTTCCACTCTCC--AAACTTGGTGGTGTAT-						
	220	230	240	250	260	270	280
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	ATCAGCCAAGAGAAATTTGAGAAATCTAGAGCCTATAGTCTTGATAAAAGCCACTTCCATTTTGTTCACG						
PPI-SoyCPP	ATCAGCCAAGAGAAATTTGAGAAATCTAGAGCCTATAGTCTTGATAAAAGCCACTTCCATTTTGTTCACG						
afc1	-----						
AT4g01320	-----						
AF007269	CCTTGATCTGAGGTTTATTACTCCTACTAGTTTCTTGTCTTACTCGTG--CGTTT-GATTTGATTGAG						
Consensus	---AGCCAAGAGAAGTTTGAGAAATCTGAG--CTACAGTCTTGAAAAG--CATT--CATTT-GTTCA-G						
	290	300	310	320	330	340	350
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	AGTTTGTGACAATAGTGACAGACTCTACAATTTTGTACTTTGGGGTATTGCCCTGGTTTGGAA----G						
PPI-SoyCPP	AGTTTGTGACAATAGTGACAGACTCTACAATTTTGTACTTTGGGGTATTGCCCTGGTTTGGAA----G						
afc1	-----						
AT4g01320	-----						
AF007269	CTTATGTGA-TTTCATCATCTCTCCTCGGTTTGTAGAAATGTACGGAGCTTCTCTGTTAACCAAAATCTAG						
Consensus	AGTTTGTGA--CATAGT--TAGACTCT-CAATTTTGT-CTTGGG--TTTGCCTGGTTTGGAA----G						
	360	370	380	390	400	410	420
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	AAATCAGGAGATTTTATGACAATAGCTGGTTTCAATGCTGAGAAATGAAATACTGCATACCCTTGCCTTCT						
PPI-SoyCPP	AAATCAGGAGATTTTATGACAATAGCTGGTTTCAATGCTGAGAAATGAAATACTGCATACCCTTGCCTTCT						
afc1	-----						
AT4g01320	-----						
AF007269	GATTGGGAAGAAAAGTCGGAGTCTTTTTTTTCTCATTCCCGATTGGAAATTGAGAATCTTGAAATTTT						

Consensus AT-TCGGG---TTTGTCAA-----TTGGT-----CATCGAGAATGAAAT-CTGCATACC-TT---CTTCT

430 440 450 460 470 480 490

PPI-AtCPP NA TAGCAGGGCTGATGATTTGGTCAAGATAACAGATTGCCCCCTTTCTCTGTACTCAACTTTT---GTG

PPI-BnCPP TAGCAGGGCTGATGATTTGGTCAAGATAACAGATTGCCCCCTTTCTCTGTACTCAACTTTT---GTG

PPI-SoyCPP TAGCAGGGCTGATGATTTGGTCAAGATAACAGATTGCCCCCTTTCTCTGTACTCAACTTTT---GTG

afcl

AT4g01320 TCTTTGTTCAAGTCATACAGCTTGAGGTTTGGGTTTCTTGTGAGGTTATTATTATGTTTCGTGACTGCA

AF007269 TCTTTGTTCAAGTCATACAGCTTGAGGTTTGGGTTTCTTGTGAGGTTATTATTATGTTTCGTGACTGCA

Consensus T-GCGGT---ATGAT--GGTCAAGATA--CGATTGCCCCCTTTCTT--GTACTCAACTTT-----GTG

500 510 520 530 540 550 560

PPI-AtCPP NA ATTGAGGCCCGTCATGGTTTAAATAAGCAAACAC--CATGGTTATTCTTTAGGGACATGCTTAAAGGAAT

PPI-BnCPP ATTGAGGCCCGTCATGGTTTAAATAAGCAAACAC--CATGGTTATTCTTTAGGGACATGCTTAAAGGAAT

PPI-SoyCPP ATTGAGGCCCGTCATGGTTTAAATAAGCAAACAC--CATGGTTATTCTTTAGGGACATGCTTAAAGGAAT

afcl

AT4g01320 ACTAGAGTTTCTGAGTTTGTGAAATGGGTTTGTGTTGTGGAACCGTATGTGAATGTTGCATCAAAA

AF007269 ACTAGAGTTTCTGAGTTTGTGAAATGGGTTTGTGTTGTGGAACCGTATGTGAATGTTGCATCAAAA

Consensus AT--GAGTCCG-CATGGTTAAACAAACA-----CATGGTT--TCTTAGGGACATG--TAAAGGAAT

570 580 590 600 610 620 630

PPI-AtCPP NA TTTCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAA-----TCATTGTAA-TAGTACAGAAAGG

PPI-BnCPP TTTCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAA-----TCATTGTAA-TAGTACAGAAAGG

PPI-SoyCPP TTTCTTTCTGTAATAATTGGTCCACCTATTGTGGCTGCAA-----TCATTGTAA-TAGTACAGAAAGG

afcl

AT4g01320 CTCTTTCAGTGCTCCAATGTTTCCATCAGTAGTCAGCACAAAGAGATCTTTTATATCTGGTTGATCAAAA

AF007269 CTCTTTCAGTGCTCCAATGTTTCCATCAGTAGTCAGCACAAAGAGATCTTTTATATCTGGTTGATCAAAA

Consensus TTCTTCTGTATA-----G--CC-TATTGTG-CTGCAA-----T-ATTGTA--TAGT-CAGAAAGG

640 650 660 670 680 690 700

PPI-AtCPP NA AGGTCCATA--CTTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCCTT

PPI-BnCPP AGGTCCATA--CTTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCCTT

PPI-SoyCPP AGGTCCATA--CTTGGCCATCTATCTTTGGGTTTTTACGTTTGGTCTTTCTATTGTGATGATGACCCCTT

afcl

AT4g01320 AAGTAGATGATGTTATTGAATTTTCAAGTGATGAGTATCTGTTGTTGTGGCATTAGAGTAGATTCGTAT

AF007269 AAGTAGATGATGTTATTGAATTTTCAAGTGATGAGTATCTGTTGTTGTGGCATTAGAGTAGATTCGTAT

Consensus AGGTCC-----TATG-CCATCTATCTTGGG-----TTTAGTTTTCTTCTTGTGATGATGACC--TT

710 720 730 740 750 760 770

PPI-AtCPP NA ATCCAGTACTA-ATAGCTCCACTCTTCAATAAGTTCACTCCACT--TCCAGATGGTCAACTCAGGGAGAA

PPI-BnCPP ATCCAGTACTA-ATAGCTCCACTCTTCAATAAGTTCACTCCACT--TCCAGATGGTCAACTCAGGGAGAA

PPI-SoyCPP ATCCAGTACTA-ATAGCTCCACTCTTCAATAAGTTCACTCCACT--TCCAGATGGTCAACTCAGGGAGAA

afcl

AT4g01320 GCGGATTCCT--TTCATGGAAACCGCTGTTGGTTTTATGATAGTATGATGACATTTTGAGACGTATTTTGG

AF007269 GCGGATTCCT--TTCATGGAAACCGCTGTTGGTTTTATGATAGTATGATGACATTTTGAGACGTATTTTGG

Consensus A-CC-----GTTATGCCCTCTTCAA-AAGTTCACTCC-CT--TCCAGATGG--ACTC-GGGAGAA

780 790 800 810 820 830 840

PPI-AtCPP NA AATCGAGA-AACTTGCCTTCTCCT-CAACTATCCGTTAAAGAAACTATTGTTGTCGATGGATCCACAA

PPI-BnCPP AATCGAGA-AACTTGCCTTCTCCT-CAACTATCCGTTAAAGAAACTATTGTTGTCGATGGATCCACAA

PPI-SoyCPP AATCGAGA-AACTTGCCTTCTCCT-CAACTATCCGTTAAAGAAACTATTGTTGTCGATGGATCCACAA

afcl

AT4g01320 ATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCCGAAAAC-TTGGTTGGTG-TAATTAGCCAA

AF007269 ATCTGAGGCAACTCACTGCTCTCAAGCTTCCAACTCTCCCGAAAAC-TTGGTTGGTG-TAATTAGCCAA

Consensus A--TGAGA-AACTTGCCTTCTCCT---A---ATCTTAAAG--AACTATTGTTGTCGATGGATC-ACAA

850 860 870 880 890 900 910

PPI-AtCPP NA GATCAAGTCACAGCAATGCCTATATGTATGGATTCTTCAAGAACAGAGGATTGTCC---CTTATGACAC

PPI-BnCPP G-AGAAGTTTGAGAAATCTCGA-GCTTACAG-----

PPI-SoyCPP GATCAAGTCACAGCAATGCCTATATGTATGGATTCTTCAAGAACAGAGGATTGTCC---CTTATGACAC

afcl

AT4g01320 G-AGAAGTTTGAGAAATCAGG-GCATAACAG-----

AF007269 G-AGAAGTTTGAGAAATCAGG-GCATAACAG-----

Consensus G--TCAAG-CATAG-AAATGCTAA--TGATAG--TTCTTAAGAACAA-AGGATTGTCC---TTATGACAC

920 930 940 950 960 970 980

PPI-AtCPP NA ATTAATTC-----AACAGTGCAAAGAC-GATGAGGAAATTGTTG-CTGTTATTGCGCATGAG

PPI-BnCPP ATTAATTC-----AACAGTGCAAAGAC-GATGAGGAAATTGTTG-CTGTTATTGCGCATGAG

PPI-SoyCPP ATTAATTC-----AACAGTGCAAAGAC-GATGAGGAAATTGTTG-CTGTTATTGCGCATGAG



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afcl -----TCTTGACAAA--AGCTATTTTCACTTTG
AT4g01320 -----GGATATCATCACTGAGAACTTTAATATATGCACCTATTTTCACTTTG
AF007269 TTTAGTTTTTTATAATTGCCAGGGGATATCATCACTGAGAACTTTAATATATGCACCTATTTTCACTTTG
Consensus ATTATTC-----ACAGTGCAA-----GAAGAAATTGTG---CTTATTGCG---AGA

          990      1000      1010      1020      1030      1040      1050
PPI-AtCPP NA TTGGGACACTGGAAGCTCAACCACTACTGTGTACACATTGTTGCTATGCAGATTCTTACACTTCTACAAT
PPI-BnCPP TTTCATGAGTTTGTACTATA-CTTATGGAAGCTGCGAT-TCGTTCCTTGGCATCTTGC---CTTGGTT
PPI-SoyCPP TTGGGACACTGGAAGCTCAACCACTACTGTGTACACATTGTTGCTATGCAGATTCTTACACTTCTACAAT
afcl TTTCATGAGTTTGTAACTATA-CTTATGGAAGCTGCGAAT-TTGTTCCTTGGCATCTTGC---CTTGGTT
AT4g01320 TTTCATGAGTTTGTAACTATA-CTTATGGAAGCTGCGAAT-TTGTTCCTTGGCATCTTGC---CTTGGTT
AF007269 TTTCATGAGTTTGTAACTATA-CTTATGGAAGCTGCGAAT-TTGTTCCTTGGCATCTTGC---CTTGGTT
Consensus GTGGGACACTGGA-----CTAACCACTTACACATT-ATTGCTTC---AATCTT---CTTACAAAT

          1060      1070      1080      1090      1100      1110      1120
PPI-AtCPP NA TTGGAGGATATACACTAGTGCAGAAATTCAGCTGATCTGTATCGAAGCTTTGGGTTTGATACGCAGCCAGT
PPI-BnCPP TTGGAGG-----
PPI-SoyCPP TTGGAGGATATACACTAGTGCAGAAATTCAGCTGATCTGTATCGAAGCTTTGGGTTTGATACGCAGCCAGT
afcl TTGGAGG-----
AT4g01320 TTGGAGG-----
AF007269 TTGGAGGATATACATCTGGTTTCGGTATACAGTATCT-CATTTGAATATAGAGTTGTTACATTACAA--
Consensus TTGGAGGATACAC-CTAGTG--AAATCC--TGATCT---TGAG---TTGGTTTGATAC-CAGCCG--

          1130      1140      1150      1160      1170      1180      1190
PPI-AtCPP NA CCTCATTTGGGCTCATCATATTTTCAAGCACTGTAAATCCCACTTCAGCAATTTGGTCAGGTTTGGTCT---G
PPI-BnCPP -----ATATCTGGCGGC-TTCTACCAA-TGGTGGGACTCGATTCAGAG
PPI-SoyCPP CCTCATTTGGGCTCATCATATTTTCAAGCACTGTAAATCCCACTTCAGCAATTTGGTCAGGTTTGGTCT---G
afcl -----ATGTCGTGAGCT-GTTTTACCGA-CGTTGGGCTTGATTCAGAG
AT4g01320 -----ATGTCGTGAGCT-GTTTTACCGA-CGTTGGGCTTGATTCAGAG
AF007269 TTGTAAAGTTTTCATTTTACCTTAGATGTCTGGAGCT-GTTTTACCGA-CGTTGGGCTTGATTCAGAG
Consensus TCTCATTTGG---TATCATATTTCAAGCACTGTAAATCC-ACCTCA-----CATGTAGCTTTGCT-----

          1200      1210      1220      1230      1240      1250      1260
PPI-AtCPP NA AACCTAGTCAGCCGATCATTTGAATTTCAAGGCTGATGCTTTGCCAAGAAGCTTGATATGCATCTGGAT
PPI-BnCPP AATGAAATCCTGCACACTCTTTCAATCTTGGCTGGTC-TTATGACATGGTCACAG-----
PPI-SoyCPP AACCTAGTCAGCCGATCATTTGAATTTCAAGGCTGATGCTTTGCCAAGAAGCTTGATATGCATCTGGAT
afcl AATGAAATACTGCATACTCTTTCAATCTTGGCTGGTC-TTATGACATGGTCACAG-----
AT4g01320 AATGAAATACTGCATACTCTTTCAATCTTGGCTGGTC-TTATGACATGGTCACAG-----
AF007269 AATGAAATACTGCATACTCTTTCAATCTTGGCTGGTC-TTATGACATGGTCACAGGTGTTCCAAATAAAC
Consensus AACCTG-----TAGCGACTTTGATTTCAAGGCTGATG-CTTTGC---GAAGCTTG-TATGCAGTCGG--

          1270      1280      1290      1300      1310      1320      1330
PPI-AtCPP NA TACGCGGTG--GTCTTGTGAAACTACAGGAGGAGAATCTGTCAGCT---ATGAATACAGATCCTTGGTA
PPI-BnCPP -----
PPI-SoyCPP TACGCGGTG--GTCTTGTGAAACTACAGGAGGAGAATCTGTCAGCT---ATGAATACAGATCCTTGGTA
afcl -----
AT4g01320 -----
AF007269 CCCTTCATATAGTCCCTATACGTTTAGCATCAAAATATCTATTTTCTTAAGATAATAATATTTCTTTTATA
Consensus --T-----GTCTAGTGAA-CTACAGGAGAGAA--TGTCAGC-----ATGAA-ACAGATCCTTG-TA

          1340      1350      1360      1370      1380      1390      1400
PPI-AtCPP NA CTCT---GCTTATCACTATTTCTCATCTCCCTT---TGTGAAAGATTGCCGCGCTGGACGAA
PPI-BnCPP -----ATCACTGATTTGTCATTTCTTTG---TACTCAACTTTCC---TGATCGAG---
PPI-SoyCPP CTCT---GCTTATCACTATTTCTCATCTCCCTT---TGTGAAAGATTGCCGCGCTGGACGAA
afcl -----ATCACTGATTTGTCATTTCTTTG---TACTCAACTTTCC---TGATCGAG---
AT4g01320 -----ATCACTGATTTGTCATTTCTTTG---TACTCAACTTTCC---TGATCGAG---
AF007269 TTCTGATGCAGATCACTGATTTGTCATTTCTTTG---TACTCAACTTTCC---TGATCGAG---
Consensus CTC---GCTTATCACTATCCACTCCCTTGTGAAAGATTGCTGAGAGAAAGAAGAGATAATCTAATTCT

          1410      1420      1430      1440      1450      1460      1470
PPI-AtCPP NA --CCGGATAAGAAGGAAGACTAAE-----
PPI-BnCPP --TCTCGGCATGGGTTCAACAAA-----
PPI-SoyCPP --CCGGATAAGAAGGAAGACTAA-----
afcl --TCTCGGCATGGGTTCAACAAA-----
AT4g01320 --TCTCGGCATGGGTTCAACAAA-----
AF007269 --TCTCGGCATGGGTTCAACAAAGTATGTCGATTTTCCAACACTACCTTGTGACTTACGTTTTTTTATCA
Consensus TTCTTTTTCATGGAGGTAAACAAAGTATGTCGATTTTCCAACACTACCTTGTGACTTACGTTTTTTTATCA

          1480      1490      1500      1510      1520      1530      1540
PPI-AtCPP NA -----
PPI-BnCPP -----
PPI-SoyCPP -----
afcl -----
AT4g01320 -----
AF007269 -----
Consensus -----
```

PPI-AtCPP NA	-----
PPI-BnCPP	-----CAAACAATATGGATGTTTCATTAGGGACATGA-----
PPI-SoyCPP	-----
afcl	-----CAAACAATATGGATGTTTCATTAGGGACATGA-----
AT4g01320	-----CAAACAATATGGATGTTTCATTAGGGACATGA-----
AF007269	GAGATGTGGATTAAATTTGCTTCTAAATCTGTTGACAGCAAACAATATGGATGTTTCATTAGGGACATGA
Consensus	GAGATGTGGATTAAATTTGCTTCTAAATCTGTTGACAGCAAACAATATGGATGTTTCATTAGGGACATGA
	1550 1560 1570 1580 1590 1600 1610
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	TCAAAGGAATATCTCTCTCTGTCTATAGCTGCCCCCTCTATCTTGGCCGCAATTATTGTTATAGTTCAG--
PPI-SoyCPP	-----
afcl	TCAAAGGAATATCTCTCTCTGTCTATAGCTGCCCCCTCTATCTTGGCCGCAATTATTGTTATAGTTCAG--
AT4g01320	TCAAAGGAATATCTCTCTCTGTCTATAGCTGCCCCCTCTATCTTGGCCGCAATTATTGTTATAGTTCAG--
AF007269	TCAAAGGAATATCTCTCTCTGTCTATAGCTGCCCCCTCTATCTTGGCCGCAATTATTGTTATAGTTCAGGT
Consensus	TCAAAGGAATATCTCTCTCTGTCTATAGCTGCCCCCTCTATCTTGGCCGCAATTATTGTTATAGTTCAGGT
	1620 1630 1640 1650 1660 1670 1680
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	-----
PPI-SoyCPP	-----
afcl	-----
AT4g01320	-----
AF007269	TTGATGATTCTGGATTTCATCTTATTTCTGAGTTTTTACATGGATGACTATTCTCCATTGAGTGTGAGCT
Consensus	TTGATGATTCTGGATTTCATCTTATTTCTGAGTTTTTACATGGATGACTATTCTCCATTGAGTGTGAGCT
	1690 1700 1710 1720 1730 1740 1750
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	-----
PPI-SoyCPP	-----
afcl	-----
AT4g01320	-----
AF007269	TCAAAGTTTTTAGTTTTCTGTTTAAAAATTTAAAAATTTGCTTCTCTGAGCATGAAGTTTTCTATCTTTTTTC
Consensus	TCAAAGTTTTTAGTTTTCTGTTTAAAAATTTAAAAATTTGCTTCTCTGAGCATGAAGTTTTCTATCTTTTTTC
	1760 1770 1780 1790 1800 1810 1820
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	---AAAGGAGGTCCCTTACCTCGCCATCTATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGA
PPI-SoyCPP	-----
afcl	---AAAGGAGGTCCCTTATCTTGCCATCTATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGA
AT4g01320	---AAAGGAGGTCCCTTATCTTGCCATCTATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGA
AF007269	CAGAAAGGAGGTCCCTTATCTTGCCATCTATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGA
Consensus	CAGAAAGGAGGTCCCTTATCTTGCCATCTATCTGTGGGCATTTCATGTTTATCCTGTCTCTAGTGATGATGA
	1830 1840 1850 1860 1870 1880 1890
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	CTATATACCCGGTTTGATTGCACCGCTTTTCAACAAGTTCACTCCT
PPI-SoyCPP	-----
afcl	CTATATACCCGGTTTGATTGCACCGCTTTTCAACAAGTTCACTCCT
AT4g01320	CTATATACCCGGTTTGATTGCACCGCTTTTCAACAAGTTCACTCCT
AF007269	CTATATACCCGGTTTGATTGCACCGCTTTTCAACAAGTTCACTCCTGTGTGTATTTCTGTCTATGGCCAT
Consensus	CTATATACCCGGTTTGATTGCACCGCTTTTCAACAAGTTCACTCCTGTGTGTATTTCTGTCTATGGCCAT
	1900 1910 1920 1930 1940 1950 1960
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	-----
PPI-SoyCPP	-----
afcl	-----
AT4g01320	-----
AF007269	TTTACAATTCAGTCTTGTGTTGTCATATGTTGTACCAGACAATATAATCTCCCGCTTTTTTATGGCTATA
Consensus	TTTACAATTCAGTCTTGTGTTGTCATATGTTGTACCAGACAATATAATCTCCCGCTTTTTTATGGCTATA
	1970 1980 1990 2000 2010 2020 2030
PPI-AtCPP NA	.... .... .... .... .... .... .... .... .... .... .... ....
PPI-BnCPP	-CTTCCTGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCCTTGAAGAAG
PPI-SoyCPP	-----
afcl	-CTTCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCCTTGAAGAAG
AT4g01320	-CTTCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCCTTGAAGAAG
AF007269	GCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCCTTGAAGAAG
Consensus	GCTTCCAGATGGAGACCTCCGGGAGAAGATTGAGAACTTGCTTCTTCTCTAAAGTTTCCTTGAAGAAG

	2040	2050	2060	2070	2080	2090	2100
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	CTGTTTGTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG						
PPI-SoyCPP	CTGTTTGTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG						
afcl	CTGTTTGTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG						
AT4g01320	CTGTTTGTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATG						
AF007269	CTGTTTGTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAGAAGCTTGAGATCTCTTCTACCT						
Consensus	CTGTTTGTGTGTCGATGGATCTACAAGGTCAAGCCATAGCAATGTGAGAAGCTTGAGATCTCTTCTACCT						
	2110	2120	2130	2140	2150	2160	2170
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	..... ..... ..... ..... ..... ..... CTTACATGTATGGTTTC						
PPI-SoyCPP	..... ..... ..... ..... ..... ..... CTTACATGTATGGTTTC						
afcl	..... ..... ..... ..... ..... ..... CTTACATGTATGGTTTC						
AT4g01320	..... ..... ..... ..... ..... ..... CTTACATGTATGGTTTC						
AF007269	ACTTTACTCTAGTTTACCATTAGAAGCTTACGTATCTTGTACATCATAACAGCTTACATGTATGGTTTC						
Consensus	ACTTTACTCTAGTTTACCATTAGAAGCTTACGTATCTTGTACATCATAACAGCTTACATGTATGGTTTC						
	2180	2190	2200	2210	2220	2230	2240
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	TTCAAGAACAAAAGGATTGTTCTTTATGATACGTTGATTTCAGCAG						
PPI-SoyCPP	TTCAAGAACAAAAGGATTGTTCTTTATGATACGTTGATTTCAGCAG						
afcl	TTCAAGAACAAAAGGATTGTTCTTTATGATACGTTGATTTCAGCAG						
AT4g01320	TTCAAGAACAAAAGGATTGTTCTTTATGATACGTTGATTTCAGCAG						
AF007269	TTCAAGAACAAAAGGATTGTTCTTTATGATACGTTGATTTCAGCAGTACTGTGACTCTTGATGCTTCAAA						
Consensus	TTCAAGAACAAAAGGATTGTTCTTTATGATACGTTGATTTCAGCAGTACTGTGACTCTTGATGCTTCAAA						
	2250	2260	2270	2280	2290	2300	2310
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	..... ..... ..... ..... ..... ..... TGCAGAAAT						
PPI-SoyCPP	..... ..... ..... ..... ..... ..... TGCAGAAAT						
afcl	..... ..... ..... ..... ..... ..... TGCAGAAAT						
AT4g01320	..... ..... ..... ..... ..... ..... TGCAGAAAT						
AF007269	CGAGCTATACTCACATTTCTGTTTCTGGTCTGAAACATAACATAATCTTCTATTGTGCAGTGCAGAAAT						
Consensus	CGAGCTATACTCACATTTCTGTTTCTGGTCTGAAACATAACATAATCTTCTATTGTGCAGTGCAGAAAT						
	2320	2330	2340	2350	2360	2370	2380
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	GAGCATGAAATTTGTGGCGGTATTGACACGAGCTTGGACATGGAAACTGAATCACACTACATACTCGT						
PPI-SoyCPP	GAGCATGAAATTTGTGGCGGTATTGACACGAGCTTGGACATGGAAACTGAATCACACTACATACTCGT						
afcl	GAGCATGAAATTTGTGGCGGTATTGACACGAGCTTGGACATGGAAACTGAATCACACTACATACTCGT						
AT4g01320	GAGCATGAAATTTGTGGCGGTATTGACACGAGCTTGGACATGGAAACTGAATCACACTACATACTCGT						
AF007269	GAGCATGAAATTTGTGGCGGTATTGACACGAGCTTGGACATGGAAACTGAATCACACTACATACTCGT						
Consensus	GAGCATGAAATTTGTGGCGGTATTGACACGAGCTTGGACATGGAAACTGAATCACACTACATACTCGT						
	2390	2400	2410	2420	2430	2440	2450
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	TCATTGCAAGTTCAA						
PPI-SoyCPP	TCATTGCAAGTTCAA						
afcl	TCATTGCAAGTTCAA						
AT4g01320	TCATTGCAAGTTCAA						
AF007269	TCATTGCAAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACTTACTCACATCTACATTTCACTTAAAGAAA						
Consensus	TCATTGCAAGTTCAAGTGAGGCTCAACCGACAGTTCAAAAACTTACTCACATCTACATTTCACTTAAAGAAA						
	2460	2470	2480	2490	2500	2510	2520
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	..... ..... ..... ..... ..... ..... ATCCTTGCCTTCTTCAATTTGGAGGATACAC						
PPI-SoyCPP	..... ..... ..... ..... ..... ..... ATCCTTGCCTTCTTCAATTTGGAGGATACAC						
afcl	..... ..... ..... ..... ..... ..... ATCCTTGCCTTCTTCAATTTGGAGGATACAC						
AT4g01320	..... ..... ..... ..... ..... ..... ATCCTTGCCTTCTTCAATTTGGAGGATACAC						
AF007269	TCATGTCTTATGACCTCTCTCAATGTTTTGCTTGCAGATCCTTGCCTTCTTCAATTTGGAGGATACAC						
Consensus	TCATGTCTTATGACCTCTCTCAATGTTTTGCTTGCAGATCCTTGCCTTCTTCAATTTGGAGGATACAC						
	2530	2540	2550	2560	2570	2580	2590
PPI-AtCPP NA	..... ..... ..... ..... ..... ..... ..... .....						
PPI-BnCPP	TCTTGTGAGAACTCCACTGATCTCTTCAGGAGTTTTCGTTTGTATACACACCAAGTTCTCATTGGTTTC						
PPI-SoyCPP	TCTTGTGAGAACTCCACTGATCTCTTCAGGAGTTTTCGTTTGTATACACACCAAGTTCTCATTGGTTTC						
afcl	TCTTGTGAGAACTCCACTGATCTCTTCAGGAGTTTTCGTTTGTATACACACCAAGTTCTCATTGGTTTC						

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PPI-BnCPP      TCCTCTTGTAGAGAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA-
PPI-SoyCPP     TCCTCTTGTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA-
afcl           TCCTCTTGTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA-
AT4g01320      TCCTCTTGTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA-
AF007269       TCCTCTTGTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA-
Consensus      TCCTCTTGTGAAAGGCTTCGAGCCATTGATGGAGAAGACAAGAAGACAGATTAA-

```

Table XX. ClustalW Analysis of PPI/Generic Nucleic Acids

- 1) PPI-AtCPP (SEQ ID NO:97)
- 2) PPI-BnCPP (SEQ ID NO:109)
- 3) PPI-SoyCPP (SEQ ID NO:112)
- 4) afcl (SEQ ID NO:124)
- 5) AT4g01320 (SEQ ID NO:126)
- 6) AF007269 (SEQ ID NO:128)
- 6) Consensus (SEQ ID NO:170)

```

          10      20      30      40      50      60      70
PPI-AtCPP NA .....|.....|.....|.....|.....|.....|.....|
PPI-BnCPP .....|.....|.....|.....|.....|.....|.....|
PPI-SoyCPP .....|.....|.....|.....|.....|.....|.....|
afcl .....|.....|.....|.....|.....|.....|.....|
AT4g01320 .....|.....|.....|.....|.....|.....|.....|
AF007269 ATGGCGATTCCCTTCATGGAAACCGTCGTGGGTAAGCTTCAAACCTTTTCTGAGACATTTACTATCC

```

Table 38. ClustalW Analysis of PPI/Generic Amino Acids

- 1) PPI-AtCPP (SEQ ID NO:98)
- 2) PPI-BnCPP (SEQ ID NO:110)
- 3) PPI-SoyCPP (SEQ ID NO:113)
- 4) afcl (SEQ ID NO:125)
- 5) AT4g01320 (SEQ ID NO:127)
- 6) AF007269 (SEQ ID NO:129)
- 7) Consensus Gener (SEQ ID NO:169)

```

          10      20      30      40      50      60
PPI-AtCPP MAIPFMEAVVVGFMIMYIFETYLDLRQHRALKLPTLPKTLGVISQEKFEKSRAYS--LD 58
PPI-BnCPP MAIPFMETVVVGFMIVMYIFETYLDLRQHTALKLPTLPKTLGVISQEKFEKSRAYS--LD 58
PPI-SoyCPP MAIPFMEAVVVGFMIMYIFETYLDLRQHRALKLPTLPKTLGVISQEKFEKSRAYS--LD 58
afcl MAIPFMETVVVGFMIVMYIFETYLDLRQHTALKLPTLPKTLGVISQEKFEKSRAYS--LD 58
AT4g01320 MAIPFMETVVVGFMIVMYIFETYLDLRQHTALKLPTLPKTLGVISQEKFEKSRAYS--LD 60
AF007269 MAIPFMETVVVGFMIVMYIFETYLDLRQHTALKLPTLPKTLGVISQEKFEKSRAYS--LD 41
Consensus Gener MAIPFMEAVVVGFMIMYIFETYLDLRQXXALKLPTLPKTLXXXXXXXXXXXXXXXXXXXX 60

          70      80      90      100     110     120
PPI-AtCPP KS-----FHFVHEFVTIIVTDSITILYFGVLPWFWKKSCDFMTIAGFNAENEILHTLSFLA 113
PPI-BnCPP KS-----FHFVHEFVTIIVTDSITILYFGVLPWFWKKISCGFLPMVGLDPENEILHTLSFLA 113
PPI-SoyCPP KS-----FHFVHEFVTIIVTDSITILYFGVLPWFWKKSCDFMTIAGFNAENEILHTLSFLA 113
afcl KS-----FHFVHEFVTIIVTDSITILYFGVLPWFWKKSCDFMTIAGFNAENEILHTLSFLA 113
AT4g01320 ENFNICSFHFVHEFVTIIVTDSITILYFGVLPWFWKKSCAVLPRLGLDPENEILHTLSFLA 120
AF007269 -----TDLPPSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 41
Consensus Gener XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 120

          130     140     150     160     170     180
PPI-AtCPP GEMTWSQITDLPFSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 173
PPI-BnCPP GEMTWSQITDLPFSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 173
PPI-SoyCPP GEMTWSQITDLPFSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 173
afcl GEMTWSQITDLPFSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 173
AT4g01320 GEMTWSQITDLPFSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 180
AF007269 -----TDLPPSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 93
Consensus Gener XXXXXXXXTDLPPSLYSTFVIESRHGFNKTQIWMFIRDMIKGTFLSVILGPPIVAAILIFI 180

          190     200     210     220     230     240

```

### Example 47 Cloning, vector construction and over-expression of AtFT-B sequences in *Arabidopsis* produces a dominant-negative phenotype

Farnesyltransferase is a heterodimer formed by its  $\alpha$ - and  $\beta$ - subunits and its activity relies on the proper dimerization between these subunits. Increased ABA sensitivity can be achieved by the over-expression of a non-full-length form of AtFTB (SEQ ID NO:1) in Arabidopsis. In the corollary experiment, over-expression of the full-length AtFTB failed to alter the ABA sensitivity. These results suggest that the phenotype of enhanced ABA response is likely the result of dominant-negative effect of the truncated form AtFTB. The truncated AtFTB maybe nonfunctional or possess limited functionality *in vivo* as compared to a full length endogenous subunit. However, The reduction of Ft activity results in enhanced ABA sensitivity.

*Cloning*

The farnesyltransferase sequence described by SEQ ID NO:1 was cloned into an appropriate vector under the transcriptional control of the 35S CaMV promoter (pBI121 derived vector) in the sense orientation for expression in plant cells. This vector was designated  $\Delta$ N90AtFTB and designated SEQ ID NO:79. The protein encoded by SEQ ID NO:1 has been determined to lack the 5' 270 nucleotides, and therefore does not code for the 5' terminal 90 amino acids. The full length farnesyltransferase sequence was obtained using the primer pair identified by SEQ ID NO:86 and SEQ ID NO:171 and methodology as described elsewhere in this document. The resulting sequence, identified as SEQ ID NO:172 was cloned into an appropriate vector under the transcriptional control of the 35S CaMV promoter (pBI121 derived vector) in the sense orientation for expression in plant cells. This vector was designated pBI121-AtFTB, SEQ ID NO:173. The protein encoded by SEQ ID NO:172 has been determined to represent the full length polypeptide.

**Agrobacterium-mediated transformation, transgenic line selection and ABA test.**

Agrobacterium strain GV3101 carrying the binary constructs described above were transformed into *Arabidopsis thaliana* via agrobacterium-mediated floral dipping transformation.

Transformed *Arabidopsis* lines (T1) were selected on Murashige/Skoog (Sigma) plates containing kanamycin (50  $\mu\text{g}/\mu\text{l}$ ). Kanamycin-resistant seedlings were then transferred to soil. The subsequent T2 seeds were harvested from individual transgenic lines for ABA tests.

**Northern blot analysis.** Total RNA was isolated from two-week-old T2 *Arabidopsis* plants of the pBI121- $\Delta$ N90AtFTB, as well as from wild-type Columbia and *era1* mutant plants. After separated in the agarose gel, RNA was transferred onto the nitrocellulose membrane and was hybridized with the  $^{32}\text{P}$ -labelled  $\Delta$ N90AtFTB DNA probe.

**Over-expression of pBI121- $\Delta$ N90AtFTB, not pBI121-AtFTB resulted in enhanced ABA sensitivity:**

Transgenic plants were selected and advanced to the second generation. T2 seeds of these two constructs were subjected to ABA test using 0.0, 0.25, 0.5 and 1.0  $\mu\text{M}$  ABA in minimum MS-agarose plates. Of the fifteen pBI121- $\Delta$ N90AtFTB lines ten showed an enhanced ABA sensitivity phenotype. At 0.5  $\mu\text{M}$  ABA, the seeds would germinate, however, the

development of the seedlings for these 10 lines were retarded or arrested, showing a typical ABA hypersensitive response. In contrast, of the fifteen pBI121-AtFTB transgenic lines, all but one line showed normal wild-type like ABA response to seed germination and seedling development.

Northern blot analysis indicated that in the transgenic lines of pBI121- $\Delta$ N90AtFTB, the expression levels were higher than the endogenous AtFTB transcript level as depicted by the wild-type control. This indicates the ABA hypersensitive phenotype of these transgenic lines is unlikely due to transcriptional co-suppression. The enhanced ABA response correlates with the results of other methods of AtFTB down-regulation, such as anti-sense and RNAi, hairpin constructs. It is possible that the observed ABA hypersensitive response in  $\Delta$ N90AtFTB transgenic lines are due to a dominant negative effect. The high transcript levels of  $\Delta$ N90AtFTB should produce an abundance of the truncated form of AtFTB which may bind to the endogenous AtFTA and result in competitive inhibition of AtFTase activity.

Further support for the interaction of truncated FT-B with endogenous FT-A comes from a yeast two-hybrid interaction experiment. Use of the  $\Delta$ N90AtFTB cDNA as bait, identified interacting clones the majority of which were found to encode FT-A.

#### SEQ ID NO:79 pBI121- $\Delta$ N90AtFTB Truncated FT-B Vector

gtttaccgcgccaatatatcctgtcaaacactgatagtttaaactgaaggcgggaaacgacaatctgatcatgagcgg  
agaattaaggaggtcacgttatgacccccgccgatgacgcgggacaagccgttttacgtttggaactgacagaaccg  
caacgtttgaaggagccactcagccgcgggtttctggagtttaattgagctaagcacatacgtcagaaaccattattgc  
gcgttcaaaagtcgcctaagggtcactatcagctagcaaatatttcttgtcaaaaatgctccactgacgttccataaa  
ttccctcgggtatccaattagagtctcatattcactctcaatccaaataatctgcacccggatctggatcgtttcgca  
tgattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggctattcggctatgactgggcacaa  
cagacaatcggctgctctgatgccgcggtgttccggctgtcagcgcagggggcgcccggttcttttgtcaagaccga  
cctgtccgggtgcctgaatgaactgcaggacgaggcagcgcggctatcgtggctggccacgacgggcgttccctgcg  
cagctgtgctcgacgttctcactgaagcgggaagggaactggctgctattgggcgaagtgcggggcaggatctcctg  
tcatctcaccttgctcctgccgagaaagtatccatcatggctgatgcaatgcccgggctgcatacgttgcggtcggc  
tacctgcccattcgaccaccaagcgaaacatcgcatcgagcgcagcgtactcggatggaagccggtcttgtcgatc  
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ggcgatgatctcgtcgtgacccatggcgatgcctgcttgcgaatatcatgggtggaaaatggcgcgttttctggatt  
catcgactgtggccggctgggtgtggcggacgcgtatcaggacatagcgttgggtacccgctgatattgctgaagagc  
ttggcggcgaatgggctgacgcgttccctcgtgctttacgggtatcgcgcgtcccgattcgcagcgcacgccttctat  
cgcttcttgacgagttcttctgagcgggactctggggttcgaaatgaccgaccaagcgacgcccacctgccatca  
cgagatttcgattccaccgcgccttctatgaaaggttgggcttcggaatcgttttccgggacgcgggctggatgat  
cctccagcgcggggatctcatgctggagtcttctcgccacgggatctctgcggaacaggcggctcgaaggtgccgata  
tcattacgacagcaacggccgacaagcacacgccacgatcctgagcgacaatatgatcggggccggcgctccacatc  
aacggcgtcggcggcgactgccaggaagaccgagatgcaccgcgatattcttgcgtgcgttcggatatttctgtgga  
gttcccgccacagaccggatgatccccgatcgttcaaacatttggcaataaagtctttaaagattgaatcctgttg  
ccggtcttgcgatgattatcataataattctgttgtaattacgttaagcatgtaataataacatgtaatgcatgacg  
ttatttatgagatgggtttttatgattagagtcccgcaattatacatttaatacgcgatagaaaacaaaatatagcg  
cgaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcgggcctcctgtcaatgctggcggcggc  
tctgggtgggtggttctgggtggcggctctgaggggtgggtggtctctgaggggtggcgggtctctgagggg



aggcgggttcgggtgggtggtctctgggttcgggtgattttgattatgaaaagatggcaaacgctaataaggggggctatga  
 ccgaaaatgccgatgaaaacgcgctacagctctgacgctaaaggcaaaccttgattctgtcgctactgattacgggtgct  
 gctatcgatgggtttcatgtgtgacgtttccggccttgctaattggtaatggtgctactgggtgattttgctgggtctaa  
 tccccaaatgggtcaagtcgggtgacgggtgataattcacctttaatagaataatttccgtcaatatttaccttccctcc  
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 taatgcagctggcagcagaggtttcccgactggaagcgggcagtgagcgcaacgcaattaatgtgagttagctcac  
 tcattaggcaccgccaggctttacactttatgcttccggctcgatgttgtgtggaattgtgagcggataacaatttc  
 acacaggaaacagctatgaccatgattacgccaagcttgcagtcctgcagcccaagatgggttagagagggttacgc  
 agcaggtctcatcaagacgatctaccggagcaataatctccaggaaatcaaataccttcccaagaagggttaaagatg  
 cagtcaaaagatttcaggactaactgcatacaagaacacagagaaagatatatttctcaagatcagaagtaactattcca  
 gtatggacgattcaaggcttgccttcacaaaccaaggcaagtaataagagattggaggtctctaaaaagggtagttccac  
 tgaatcaaaggccatggagtcaggatcaaatgacaagaagacctaacagaactcgccgtaaaagactggcgaacagttca  
 tacagagtcctcttacgactcaatgacaagaagaaaatcttcgtcaacatgggtggagcagcacacttgtctactcc  
 aaaaatatcaagatacagtcctcagaagaccaaagggaatttgagacttttcaacaaagggttaatatccggaaacct  
 cctcggattccattgcccagctatctgtcactttattgtgaagatagtggaagggaagggtgggtcctacaaatgcc  
 atcattgcgataaaaggaaaggccatcggtggaagatgcctctgcccagcagtggtcccaagatggacccccaccacg  
 agggagcatcggtggaaaaagaagacgttccaaccacgtcttcaaaagcaagtggattgatgtgatattctccactgacgt  
 aagggtgacgcacaatcccactatccttcgcaagacccttccctctatataaggaagttcattttcatttggagagaaca  
 cgggggactctagaGGATCCgtccggaattccgggtcgaccacgcgtccgggagattcagcgagataagcaattggattatcg  
 atgaaaggcttaaggcagcttgggtccgagttttctccttagatgctaactgcacttggcttgttactggattcttattcaatagcttggcttgg  
 ggagactgtggatgatgaattagaagcaatgccattgacttccctggacgctgccaggggtctgaagggtggatacgggtgggtcctggc  
 caacttccacatcttgaactacttatgtcagtgatgcacttgtactttaggaggtgacaaagcccttctcaattaaagagaaaaatg  
 tctgtttttaagacggatgaaggatacaagtgagggttcaggatgcagatgaggaagaaatggatgttcgtgcagctacactgcaatttc  
 ggttgcaagcatcctaaatattatggatgatgaactcaccagggcctaggagattacatcttgagttgccaacttatgaagggtggcattgg  
 aggggaacciggtccgaagctcacgggtgggtatacctactgtgtgttgggtgctatgattttaatcaatgaggtcgaccgttgaatttggatt  
 cattaatgaattgggtgtacatcgacaaggagtagaaatgggattcaaggtaggacgaacaaattggtcgatgggtgtcacacatttggc  
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 actcttgttccaaactggaacaacactcaaccctatctcgggctattcttttgattataaagggttttgcgcat  
 cgggaaccacatcaaacaggattttcgccgtgctggggcaaacacagcgtggacgcttgcagcaactctcagggcc  
 aggcggtgaagggaatcagctgttgcgcgtctcactggtgaaaagaaaaaacccccagtaacattaaaaacgtccg  
 caatgtgttattaagtgtgctaagcgtcaatttgtttacaccacaataatatcctgcca

SEQ ID NO:86 FORWARD Primer SacI site 5' aaacCCGGGatgcccagtagtaacccgc 3'

SEQ ID NO:171 REV Primer BamHI site 5' aaaggatcctcatgctgcttttaagaagaactcgat 3'

SEQ ID NO:172 Full length FT-B

cccgggatgccagtagtaacccgcttgattcgtttgaagtgtgtagggtcagacttgaccggagtgagctcaatcg  
 gcgaatctgtcacggaggacacgggggaatcaacgcggcgagagtgatggaagagctttcaagcctaaccgtgagtc  
 agcgcgagcaatttctgggtggagaaagatgtgttcgggatctataattacttcgacgccagcgcagctttctactcaa  
 aaatacatgatggagattcagcgagataagcaattggattatctgatgaaaggcttaaggcagcttgggtccgagctt  
 ttcttcccttagatgctaactcgaccttggctttgttactggattcttcatcattagctttgcttggggagactgtgg  
 atgatgaattagaagcaatgccattgacttcccttgacgctgccaggggtctgaagggtggatacgggtgggtggtcct

ggccaacttccacatcttgcaactacttatgtctgcagtgaatgcacttgttacttttaggaggtgacaaagccctttc  
 ttcaattaatagagaaaaaatgtctgttttttaagacggatgaaggatacaagtgagggtttcaggatgcatgata  
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 cagagacaagccgaggaaaccccggtgacttctaccacacatgttactgctgagcgggttgtctgtgggtcagcacg  
 cttgggttaaaagacgaggacactcctcctttgactcgcgacattatgggtgggtactcgaatctccttgaacctgtt  
 caacttcttcacaacattgtcatggatcagtataatgaagctatcgagttcttctttaagcagcatgaggatcc

SEQ ID NO:177 Full Length FT-B amino acid sequence encoded by SEQ ID NO:172  
 MPVVTRLIRLKCGLRLDRSGLNRRICHGGHGESTRRRVMEELSSLTVSQREQFLVENDVFGIYNYFDASDVSTQKY  
 MMEIQRDQLDYLMKGLRQLGPFSSLDANRPWLCYWLHSIALLGTVDDLELSNAIDFLGRCQSGEGGYGGGPGQ  
 LPHLATTYAAVNALVTLLGGDKALSSINREKMSCFLRMKDTSGGFRMHDMGEMDVRACYTAISVASILNIMDELDTQ  
 GLGDYILSCQTYEGGIGGEPGSEAHGGYTYCGLAAMILINEVDRLNLDLSLMNWAVHRQGVEMGFQGRTNKLVDCYT  
 FWQAAPCVLLQRLYSTNDHVDVHGSSHSISEGTNEEHHAHDEDDLEDSDDDDDSDENDEDSVNGHRIHHTSTYINRRM  
 QLVFDSLGLQRYVLLCSKIPDGGFRDKPRKPRDFYHTCYCLSGLSVAQHAWLKDEDTPLPLTRDIMGGYSNLLPEVQL  
 LHNIVMDQYNEAIEFFFKAA

### SEQ ID NO:173 pBI121-AtFTB (Full length vector Over-expression)

gtttacccgccaatatatcctgtcaaacactgatagtttaaaactgaaggcgggaaacgacaatctgatcatgagcgg  
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 caacgttgaaggagccactcagccgcgggtttctggagtttaatgagctaagcacatacgtcagaaccattattgc  
 gcgtcaaaaagtcgcctaagggtcactatcagctagcaaatatttcttggtcaaaaatgtccactgacgttccataaaa  
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 tgattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggtctatccggctatgactgggcacaa  
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 cctgtccgggtgcctgaatgaactgcaggacgaggcagcgcgggtctatcggtgggtggccacgacgggcttcccttgcg  
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 tcattacgacagcaacggccgacaagcacaacgccacgatcctgagcgacaatatgatcgggcccgggtccacatc  
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 cgaaaactaggataaattatcgcgcgcggtgtcatctatgttactagatcgggcctcctgtcaatgctggcgcggc  
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 cctcggattccattgcccagctatctgtcactttattgtgaagatagtggaaggaaggtgggtcctacaaatgcc  
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 aggagcatcgtggaaaaagaagacgttccaaccacgtcttcaaagcaagtggattgatgtgatctccactgacgt  
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 taatagcgaagaggcccgacccgatcgcccttcccaacagtgcgcagcctgaatggcgcccgctccttctgcttct  
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 ctactggtgaaaagaaaaaccacccagtagcattaaaaaacgtccgcaatgtgttattaagttgtctaagcgtcaat  
 ttgtttacaccacaatatatcctgcca

#### EXAMPLE 48 Cloning and transformation of isoprenylcysteine carboxyl methyltransferase

The *Arabidopsis* isoprenylcysteine carboxyl methyltransferase (ICMT) sequence was obtained by RT-PCR amplification using the protocol described above. The sequence was produced using the primer pair identified by SEQ ID NO:174 (5'-aaaggatccatgacagagatcttcagtgcacca-3') and SEQ ID NO:175 (5'-aaagagctctcagttcacaatggaacaccaga-3'). The sequence is identical to that reported by Accession number AB007648, GI:10177821 (Dec. 2000).

The isolated sequence was used to generate plant transformation vectors designed either to express the encoded protein or down-regulate expression. The vectors were used to transform *Arabidopsis* by the flower dipping method described elsewhere. Transformed plants were selected and propagated. Molecular and physiological analysis of the transgenic lines can be performed as detailed in other examples. Such analysis can include; molecular studies such as PCR, Southern, Northern and Western analysis; physiological analysis such as; growth studies,

tolerance to environmental stress (drought, salt, heat, cold,) tolerance to biotic stress, nutritional stress, as well as biochemical analysis.

#### SEQ ID NO:176

atgacagagatcttcagtgacaccagcatcagacagttatctcaaagtctactatcactaatcttctccacatatccgaatacattctagccatc  
accattcacggagcatcaaacgtaactcttagttcgcttttaaccacgaagcattacgcttagcaatgcttctgtcgttctcgaatacctaacg  
gagattatctcttcccggggctgaaacaacactgggtgggtcagcaactttggactcataatgatcatcggtggggaaatcatcaggaaggc  
agcgataataacagcgggaagatcggtcactcacctcataaagatcaactacgaagagcatcacgggcttgtgactcatggtgtgtatagac  
taatgaggcatccaagtactgcgggtttctcatctggtcgggcgggacacaagttatgctctgtaaccccgtttcagcagttgcgttcgcgggt  
gtcgtgtggcgggtttttgctcagagaataccgtacgaggagtattttctgaatcagtttttgggtacagtatctagagtatgcagagagtggt  
gcctctggtgttccatttgtgaactga

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All citations in this application to materials and methods are hereby incorporated by reference.

## EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

**What is claimed is:**

1. A method of producing a transgenic plant, wherein said plant has an altered phenotype selected from the group consisting of increased tolerance to stress, delayed senescence, increased ABA sensitivity, increased yield, increased productivity and increased biomass compared to a wild type plant, comprising: introducing into a plant cell a compound that inhibits farnesylation of a polypeptide having a carboxyl terminal CaaX motif to generate a transgenic cell; and regenerating a transgenic plant from said transgenic cell.
2. The method of claim 1, wherein said compound inhibits farnesyltransferase, prenylprotease, or prenylcysteine carboxyl methyltransferase expression or activity.
3. The method of claim 1, wherein said compound comprises an antisense nucleic acid sequence encoding farnesyltransferase or a portion thereof, operably linked to a promoter that is active in said plant cell.
4. The method of claim 3, wherein said promoter is selected from the group consisting of a constitutive promoter, an ABA inducible promoter, tissue specific promoters or a guard cell-specific promoter.
5. The method of claim 3, wherein said antisense nucleic acid comprises 20 or more consecutive nucleic acids complementary to any one of SEQ ID NOs: 1, 14, 40, 43, 80-85 or 172.
6. The method of claim 3, wherein said antisense nucleic acid comprises any one of SEQ ID NOs: 36, 41, or 44.
7. The method of claim 1, wherein said compound is a nucleic acid is selected from the group consisting of SEQ ID NO: 54-64.

8. The method of claim 1, wherein said compound comprises a nucleic acid molecule comprising a nucleic acid sequence encoding a mutated farnesyl transferase beta polypeptide or a fragment thereof.
9. The method of claim 1, wherein said compound comprises any one of SEQ ID NOs: 1, 14, 40, 43, 80-85, 172 or a fragment thereof.
10. The method of claim 8, wherein said nucleic acid encodes a polypeptide which is less than 314 amino acids in length.
11. The method of claim 8, wherein the nucleic acid molecule encodes a polypeptide capable of forming a dimer.
12. The method of claim 11, wherein said dimer is a heterodimer.
13. The method of claim 1, wherein said compound comprises a nucleic acid encoding a CaaX motif operably linked to a promoter.
14. The transgenic plant produced the method of claim 1.
15. The seed produced by the transgenic plant of claim 14, wherein said seed produces a plant that has an altered phenotype selected from the group consisting of increased tolerance to stress, delayed senescence, increased ABA sensitivity, increased yield, increased productivity and increased biomass compared to a wild type plant.



10	20	30	40	50	60
ATGGAGATTC	AGCGAGATAA	GCAATTGGAT	TATCTGATGA	AAGGCTTAAG	GCAGCTTGGT
70	80	90	100	110	120
CCGCAGTTTT	CTTCCTTAGA	TGCTAAGTAA	GTGACATGAT	GCTTGGCTTC	TTGTTTTTCAT
130	140	150	160	170	180
GAATTTCTTA	GTACATTTTG	TCCAGTGAGA	GAGTAAAGCT	TTGGAGCTTT	GCCAATAGAC
190	200	210	220	230	240
TTAGAAGTTT	GATTTTGGCT	TTTTGGATTT	TGGAACAGTC	GACCTTGGCT	TTGTTACTGG
250	260	270	280	290	300
ATTCTTCATT	CAATAGCTTT	GCTTGGGGAG	ACTGTGGATG	ATGAATTAGA	AAGCAATGCC
310	320	330	340	350	360
ATTGACTTCC	TTGGACGCTG	CCAGGTTAGT	CTCAATTCCT	TTTGCTTGTA	CCCAATCATG
370	380	390	400	410	420
AAAACCTCTC	ATATTTGCTC	TTGCATTCTT	CTTGATTTTC	TGCTCCTTTA	GTTTCACGTTT
430	440	450	460	470	480
TCTTTTCCCG	TTGCTATTAG	TGTTATCTGT	TATTGTTCTT	TATGTACTTA	GTTTGCTTTTC
490	500	510	520	530	540
TCATGTCGCT	TGTCAGGGCT	CTGAAGGTGG	ATACGGTGGT	GGTCCCTGGCC	AAGTAAGTAT
550	560	570	580	590	600
ATGTCTGTTT	CTTTAAAGTG	TGTGGATCAC	TTTCATTTCA	TGCAATTGGA	GAATAAACAT
610	620	630	640	650	660
TGAGACCAGA	TTATTTTATT	CTGCCAGATC	TCTTTTAGGT	GTTTTTTTTA	TGCATCATCT
670	680	690	700	710	720
CATTGTTTGG	TTGTGATGCC	TTTAATTCAA	GCAGCACACG	TAGTTTAAGT	TTAAGTTTTT
730	740	750	760	770	780
TTCTGTGAAG	ACGTAAAATG	GTGCTTTTAG	TTCAAGCAGC	ATTTAGTTGT	TTAAGTTTGT
790	800	810	820	830	840
GGTTGTAAAT	TTTCCAAACA	TGGCAGAGAA	AGTTAGGATA	TATAACTTTT	GGTCTGCCTT
850	860	870	880	890	900
TTTCAGTTTC	CTTTTTTTTT	CTACTAGTAA	TGGAGATATT	TTTTCCCAGC	TTCCACATCT
910	920	930	940	950	960
TGCAACTACT	TATGCTGCAG	TGAATGCACT	TGTTACTTTA	GGAGGTGACA	AAGCCCTTTC
970	980	990	1000	1010	1020
TTCAATTAAT	AGGTGGTGCA	TTCTTTTTTC	TTTGTGGTCA	GTTTCTTTTA	TTAAGAGTCT
1030	1040	1050	1060	1070	1080
AGTGATGTTT	CCTCTAGAAT	ACTTACATGT	GACTCATTCCT	TCTTTCAGAG	AAAAAATGTC
1090	1100	1110	1120	1130	1140
TTGTTTTTTTA	AGACGGATGA	AGGATACAAG	TGGAGGTTTC	AGGTTTGATT	CTCTTTCTGC
1150	1160	1170	1180	1190	1200
TTGAACCTCT	TAAAGGCATC	ATTTTTACTG	ACAGCGCACT	CTTTATGCAT	TCGTATCGCT
1210	1220	1230	1240	1250	1260
GTTAATGCCA	TACCTTCAGT	CATGTTGTTT	TTTAAATTCT	TGCTTAATTC	TACTTACTCA
1270	1280	1290	1300	1310	1320
CTGATCGTTA	GGATGCATGA	TATGGGAGAA	ATTGATGTTT	GTGCATGCTA	CACTGCAATT

FIGURE 1A

1330	1340	1350	1360	1370	1380
<u>TCGGTGAGTT</u>	TTACCAACTT	CTATTTTCCT	TTTCTCTGTT	TTTGTGGACA	CCAAAACTTT
1390	1400	1410	1420	1430	1440
TTAGGATTAA	TGAGATCAAC	AAAGTCTGGA	CCCATTATGC	TATGTTTCTT	CCGTTTTTCAT
1450	1460	1470	1480	1490	1500
GGCTTAAACA	TCACATTCAG	ATTACGATAT	GATCTTATTA	TTTGCACACT	TGCGCCCACC
1510	1520	1530	1540	1550	1560
AGGATACTTT	GAATAGAGAT	TACTCGTTTT	GAGACTTACA	CGTCTTGCAA	ATGCATCCTA
1570	1580	1590	1600	1610	1620
TGGCTGGTTT	TCTCCCTGAT	ATGTTTGACT	TCTCTCTTGT	GACACAGGTT	<u>GCAAGCATCC</u>
1630	1640	1650	1660	1670	1680
<u>TAAATATTAT</u>	<u>GGATGATGAA</u>	<u>CTCACCCAGG</u>	<u>GCCTAGGAGA</u>	<u>TTACATCTTG</u>	<u>AGGTAGCTTT</u>
1690	1700	1710	1720	1730	1740
TCTTATTACT	TTTATCTCGC	ATTATATATA	TATAGCTGAA	CTACTGTTAT	ACAGTTGTAA
1750	1760	1770	1780	1790	1800
ATTCAGGAAT	TCATTAATTT	CCCTGGGAAA	GCTCTTTTAA	CTCGATTTAT	ATTGAGCAGT
1810	1820	1830	1840	1850	1860
<u>TGCCAAACTT</u>	<u>ATGAAGGTGG</u>	<u>CATTGGAGGG</u>	<u>GAACCTGGCT</u>	<u>CCGAAGCTCA</u>	<u>CGGTGGGTAT</u>
1870	1880	1890	1900	1910	1920
GGTCTCCAAC	TAACTTCCAT	TATGTTGAGG	CTTAGATAAA	AATTGTGCTT	TGCTTCCCTC
1930	1940	1950	1960	1970	1980
TTCCTTGATG	ACATGGTTAT	TGATGGTTAA	GTATAATTAA	TTTTCTGAAA	TAGGATTTGT
1990	2000	2010	2020	2030	2040
CACCTGCAGC	TTGCATGCCT	GCCGCTTTGC	TTATTACCAA	GTTGTTTTTT	GTTTAGGTAT
2050	2060	2070	2080	2090	2100
<u>ACCTACTGTG</u>	<u>GTTTGGCTGC</u>	<u>TATGATTTTA</u>	<u>ATCAATGAGG</u>	<u>TCGACCCGTT</u>	<u>TGAATTGGA</u>
2110	2120	2130	2140	2150	2160
<u>TTCATTAATG</u>	GTAACATACA	ATGCTGTTTG	GAGATGATTA	ATAATTTTCC	CTGAGAGATA
2170	2180	2190	2200	2210	2220
TTTTCCTTAC	CAAATAATTT	CCTTATGATT	CTAGAATTGG	GCTGTACATC	<u>GACAAGGAGT</u>
2230	2240	2250	2260	2270	2280
<u>AGAAATGGGA</u>	<u>TTTCAAGGTA</u>	<u>GGACGAACAA</u>	<u>ATTGGTCGAT</u>	<u>GGTTGCTACA</u>	<u>CATTTTGGCA</u>
2290	2300	2310	2320	2330	2340
<u>GGTTAACTTT</u>	CTATCTTTCA	GGATTATTAT	TGGCCCTACT	TCTAAATTCT	TCACCGTTGT
2350	2360	2370	2380	2390	2400
TGTCTTTTCT	TATTTCTTTT	GGGTATATGT	TAAACAGGCA	<u>GCCCCTTGTG</u>	<u>TTCTACTACA</u>
2410	2420	2430	2440	2450	2460
<u>AAGATTATAT</u>	<u>TCAACCAATG</u>	<u>ATCATGACGT</u>	<u>TCATGGATCA</u>	<u>TCACATATAT</u>	<u>CAGAAGGGAC</u>
2470	2480	2490	2500	2510	2520
<u>AAATGAAGAA</u>	<u>CATCATGCTC</u>	<u>ATGATGAAGA</u>	<u>TGACCTTGAA</u>	<u>GACAGTGATG</u>	<u>ATGATGATGA</u>
2530	2540	2550	2560	2570	2580
<u>TTCTGATGAG</u>	<u>GACAACGATG</u>	<u>AAGGTATTCA</u>	<u>ATCAAATTTT</u>	<u>TCAACCATCA</u>	<u>AGTCCATCTG</u>
2590	2600	2610	2620	2630	2640
ATAATTCAAA	ACACAACGAA	ATTTTAGTTA	GCTTATATTT	<u>GCAGATTCAG</u>	<u>TGAATGGTCA</u>

FIGURE 1B

2650	2660	2670	2680	2690	2700
<u>CAGAATCCAT</u>	<u>CATACATCCA</u>	<u>CCTACATTAA</u>	<u>CAGGAGAATG</u>	<u>CAACTGGTTT</u>	<u>TTGATAGCCT</u>
2710	2720	2730	2740	2750	2760
<u>CGG?TTGCAG</u>	<u>AGATATGTAC</u>	<u>TCTTGTGCTC</u>	<u>TAAGGTCAGT</u>	<u>CCAGAACAAA</u>	<u>ACATCCAGTC</u>
2770	2780	2790	2800	2810	2820
<u>AAGTTAACAC</u>	<u>TTAACATTTG</u>	<u>TATAACACAA</u>	<u>GCACACACAC</u>	<u>TTGTATGCGC</u>	<u>AGATCCCTGA</u>
2830	2840	2850	2860	2870	2880
<u>CGGTGGATTC</u>	<u>AGAGACAAGC</u>	<u>CGAGGAAACC</u>	<u>CCGTGACTTC</u>	<u>TACCACACAT</u>	<u>GTTACTGCCT</u>
2890	2900	2910	2920	2930	2940
<u>GAGCGGCTTG</u>	<u>TCTGTGGCTC</u>	<u>AGCACGCTTG</u>	<u>GTTAAAAGAC</u>	<u>GAGGACACTC</u>	<u>CTCCTTTGAC</u>
2950	2960	2970	2980	2990	3000
<u>TCGCGACATT</u>	<u>ATGGGTGGCT</u>	<u>ACTCGAATCT</u>	<u>CCTTGAACCT</u>	<u>GTTCAACTTC</u>	<u>TTCACAACAT</u>
3010	3020	3030	3040	3050	3060
<u>TGTCATGGAT</u>	<u>CAGTATAATG</u>	<u>AAGCTATCGA</u>	<u>GTTCTTCTTT</u>	<u>AAAGCAGCAT</u>	<u>GACCCGTTGT</u>
3070	3080	3090	3100	3110	3120
<u>TGCTAATGTA</u>	<u>TGGGAAACCC</u>	<u>CAAACATAAG</u>	<u>AGTTTCCGTA</u>	<u>GTGTTGTAAC</u>	<u>TTGTAAGATT</u>
3130	3140	3150	3160	3170	3180
<u>TCAAAAGAAG</u>	<u>TTTCACTAAT</u>	<u>TTAACCTTAA</u>	<u>AACCTGTTAC</u>	<u>TTTTTATTAC</u>	<u>GTATA.....</u>

FIGURE 1C

MEIQRDQQLDYLMKGLRQLGPQFSSLDANRPWLCYWILHSIAL  
LGETVDDELESNAIDFLGRCQGSEGGYGGGPGQLPHLA  
TTYAAVNALVTLGGDKALSSINREKMSCFLRRMKDTSGGFR  
MHDMGEIDVRACYTAISVASILNIMDEL TQGLGDYILS  
CQTYEGGIGGEPGSEAHGGYTYCGLAAMILINEVDRLNLDL  
MNWAVHRQGVEMGFQGR TNKLVDGCYTFWQAAPCVLLQ  
RLYSTNDHDVHGSSHISEGTNEEHHAHDEDDLEDSDDDDDSD  
DNDEDSVNGHRIHHTSTYINRRMQLVFDSLGLQRYVL  
LCSEIPDGGFRDKPRKPRDFYHACYCLSGLSVAQHAWLKDED  
TPPLTRDIMGGYSNLLPEPVQLLHNI VMDQYNEAIEFFF  
KAA

FIGURE 2

10	20	30	40	50	60
CTCACTCATT	AGCACCCAG	CTTTACACTT	TATGCTTCCG	CTCGTATGTT	GTGTGGAATT
70	80	90	100	110	120
GTGAGCGATA	ACAATTTC?A	CACAGGAAAC	AGCTATGACA	TGATTACGAA	TTCAAAAAAA
130	140	150	160	170	180
TAGAGATTGG	CAATATTTTA	GTGTGTGAAT	AATATTCATC	CCTAAAAAGA	AGTCATCTTT
190	200	210	220	230	240
CGACTTTGTG	GCAACAGTTC	TGTTATTAAA	ATGTGTGAGC	GTGACATATT	TTGAAGAGGT
250	260	270	280	290	300
ACCTCGACAA	AATCGGAAGG	TGTCTCATTT	TCTTCTATCG	GAAGGCTTTC	TCGTTGAAGG
310	320	330	340	350	360
TAGTCGTTGT	AGCTGAAAAA	TTAAGAAAAC	CTAGTGAGCT	CTTCATGTAT	TCAAAAATTC
370	380	390	400	410	420
AACCAGTGTA	ATCAAACCTCA	AGAGGTAAAT	AGTTAAAATC	CCATACCAAA	CCGTGTAATC
430	440	450	460	470	480
TATGCAATAC	CTAATTAACA	AAGTTAAAAG	CGTTAGTCTA	GCAGTAATAT	TGTATCAAAA
490	500	510	520	530	540
GCTCTAACAG	TAATTAATAA	CCAGTGTCAC	CAGAAACAAA	TGTCAATAAC	ATGGAAAAAT
550	560	570	580	590	600
GAATTTAGTT	GAGTCCTGGA	GGTCGTGGAC	GTCGTGGAGG	CTGTGGACGT	CGTGAATACG
610	620	630	640	650	660
CATAAAGAAA	AATCTTATAA	TCGTGCAAAT	ATTCACCGTT	CTTCTTATAC	ATCACCTACG
670	680	690	700	710	720
GTAATAAAAG	AGTTTTATTT	CAGCAATCGT	ACATTCAAAT	TGAAACTTAG	ATACACTATA
730	740	750	760	770	780
TATTTTTTCAT	CATAACTAAC	TATAAACTAG	TCTAAACCTT	TTTTTGCTTCG	TTAGCAGAAG
790	800	810	820	830	840
CAAAGTCAAC	AGGCCATAGC	ACCTATGGAT	ACGCTTGGCG	GTTACAAAAA	GTCGAACACG
850	860	870	880	890	900
AACAACCTCT	CCAGCATCTT	TGAAGAAATT	GATGCTGTAA	CAAACAGTGT	AAGGTAAAAA
910	920	930	940	950	960
TATCAGTCAT	GCTCAGAGAA	GGAAAGTGGA	GATTGAAGAT	GGTGCTACTT	ACATATCTGA
970	980	990	1000	1010	1020
TATTTTAGTT	TGGGGAGGGA	TATGGCCATT	AAAGA?CGTC	TTTTTTGTCA	CCTGGATTTA
1030	1040	1050	1060	1070	1080
ACAGCCAAGT	GTGTTAGCAC	AAGATTCTTA	ATTGAACAGA	AATTTGTACA	AAATATCTAG
1090	1100	1110	1120	1130	1140
CAAATCCGTT	GGTTGTTTCC	TCCTGTTACA	TATGATACAA	GATCAAAGAG	TAGCCATTAG
1150	1160	1170	1180	1190	1200
AAGAAGACAG	TG?AAAGAAG	ATTGTTTTGT	CAAAGAAGAA	GAGTAATACG	AGGCCATCTT
1210	1220	1230	1240	1250	1260
AGGGTTACCT	TATTCTACTT	ATGTCTCTTG	AGAATGGAAT	TGGTCACCAA	ATCATCTTCT
1270	1280	1290	1300	1310	1320
TCAGGGTTAC	GCTTACCTAA	AAGAAGAGCA	ACAA?AAAA	AACTCTTGAG	ACAAGTTTAA

FIGURE 3A

1330	1340	1350	1360	1370	1380
CACATTAGAT	AAAAGAGAGA	GAGAGAGAGG	CAACCAAAAA	CAAACCCAAT	AAATTGCTAC
1390	1400	1410	1420	1430	1440
TAGAAGTGGC	CATGGAGAAG	ATGAAACGAG	GTTTATGTAT	TTTTCCGTTA	AGAGCAAGCA
1450	1460	1470	1480	1490	1500
ATAATATAGC	CCTAAAGAAA	TATAGACCTA	GCCTAGGAAG	AAGTTTCTAA	GACCATCCTT
1510	1520	1530	1540	1550	1560
ATCAATGAAC	TCTTACATAA	AGTTCTAAAC	AATTTTGATA	TACAAAATAA	TGTTTAAACA
1570	1580	1590	1600	1610	1620
TTAGAATGGC	TCTTACAAAA	AAAGAGAATA	AAGAAAAAAA	AAACTTAGCT	AAGAGCCATT
1630	1640	1650	1660	1670	1680
TTTCATTTCT	TAAGCACACT	TTTTTATTTT	TTTATCTTAA	TTTTATTTAA	TATAATATTT
1690	1700	1710	1720	1730	1740
TGATAGTTCT	TATGATATTG	TTAACAACCT	ATTGATAAGG	ATGCTCTAAC	TAATCTTATA
1750	1760	1770	1780	1790	1800
AATAAAACAA	TGAATCTGGT	TTGGTCTGGG	CGTAACAG?A	ATTATACTCT	TTTTTTTTTT
1810	1820	1830	1840	1850	1860
TGTCAAGAGG	AAATTATACT	AAGAAGCAAC	AGATTAAACA	TTAAAGCGTA	TAGTAAAATT
1870	1880	1890	1900	1910	1920
AATTGTTTGA	GAATCTTAAA	CCAAACCGAA	CCGGTATTAA	ACCGGAACCA	AATTGGCAAT
1930	1940	1950	1960	1970	1980
GAAATTTAGA	TGCCAGTAGT	AACCCGCTTG	ATTCGTTTGA	AGTGTGTAGG	GCTCAGACTT
1990	2000	2010	2020	2030	2040
GACCGGAGTG	GACTCAATCG	GCGAATCTGT	CACGGAGGAC	ACGGGGAATC	AACGCGGCGG
2050	2060	2070	2080	2090	2100
AGAGTGATGG	AAGAGTTTTT	AAGCCTAACC	GTGAGTCAGC	GCGAGCAATT	TCTGGTGGAG
2110	2120	2130	2140	2150	2160
AACGATGTGT	TCGGGATCTA	TAATTACTTC	GACGCCAGCG	ACGTTTCTAC	TCAAAAATAC
2170	2180	2190	2200	2210	2220
ATGTAAGCTG	ACGGATTGAT	TTTCTAGTTT	TCTTCATGAT	CTGATGAATT	TTAGTAGCGT
2230	2240	2250	2260	2270	2280
CGTGAAAGAA	TTATTTTCGT	CGATAGATGA	ATCTTACTGA	TATGGAAGTT	GTTCTATCCT
2290	2300	2310	2320	2330	2340
AGGATG	...	...	...	...	...

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FIGURE 3B

					29
Arab.	MEIQRDKQLD	YLMKGLRQLG	PQFSSLDAN-	-----	-----
Pea	..ASTAAETP	TPTVSQ.DQW	IVE.QVFHIY	QLFANIPPNA	QSII-----
Yeast	..RQRVGRSIA	RAKFINTA.L	GRKRPVMERV	VDIAHVDSSK	AIQPLMKELE
Rat	..ASSSSFTYY	CPPSSSPVWS	EPLY..RPEH	ARERLQDDSV	ETVTSIEQAK
Arab.	-----	-----	-----	-----	-----
Pea	-----	-----	-----	-----	-----
Yeast	TDTTEARYKV	LQSVLEIYDD	EKNIEPALTK	EFHKMYLDVA	FEISLPPQMT
Rat	VEEKIQEVFS	SYKFNHLVPR	LVLQREKHPH	YLKRGLRQ--	----LTDAYE
					73
Arab.	-----RPWLC	YWILHSIAL	G-ETVDDELE	SNAIDFLGRC	QGSEGGYGGG
Pea	-----	...I.....	..-SI..D..	D.TV...N..	..DPN...A..
Yeast	ALDASQ..ML	...AN.LKVM	DRDWLS.DTK	RKIV.K.FTI	SP.G.PF...
Rat	CLDAS.....	.....LE..	D-.PIPQIVA	TDVCQ..EL.	..SPD..F...
					122
Arab.	PGQLPHLATT	YAAVNALVTL	GGDKALSS-I	NREKMSCFLR	RMKDTSGG.R
Pea	...M.....	.....T.I..	..E.S.A.-	..N.LYG.M.	...QPN....
Yeast	....S...S.	...I...SLC	DNIDGCWDR.	D.KGIYQW.I	SL.EPN...K
Rat	...Y....P.	.....CII	.TEE.YNV-	....LLQY.Y	SL.QPD.S.L
					171
Arab.	-MHDMEIDV	RACYTAISVA	SILNIMDDEL	TQGLGDYILS	CQTYEGGIGG
Pea	....E.....	.....	.V...L....	IKNV...F...	.....LA.
Yeast	TCLEV..V.T	.GI.C.L.I.	TL...LTE..	.E.VLN.LKN	..N....F.S
Rat	...VG..V..	.SA.C.A...	.LT..ITPD.	FE.TAEW.AR	..NW.....
					218
Arab.	EP-GSEAHGG	YTYCGL.AM-	ILINEVDRLN	LDSLMNWAVH	RQGV-EMGFQ
Pea	..-.....	..F.....	...G..N..D	.PR.LD.V.F	...K-.C...
Yeast	C.HVD.....	..F.AT.SLA	..RSM-.QI.	VEK.LE.SSA	..LQE.R..C
Rat	V.-.M.....	..F.....LV	..KK.-RS..	.K..LQ.VTS	..MRF.G...
					267
Arab.	GRTNKLVDGC	YTFWQAAPCV	LLQR-LYSTN	DHDVHGSSHI	SEGTNEEHHA
Pea	.....	.S...GGAVA	....-H.II	.EQMAEA.QF	VTVSDAPEEK
Yeast	..S.....	.S..VGGSAA	I.EAFG.GQC	-----	-----
Rat	..C.....	.S....GLLP	..H.A.HAQG	.PALSM.---	-----
					316
Arab.	HDEDDLEDSD	DDDDSDDEDND	EDSVNGHRIH	HTSTYINRRM	-QLVFDLGL
Pea	ECL.GTSSHA	TSHIRH.GMN	.SCSSDVKNI	GYNFISEW.Q	SEPL.H.IA.
Yeast	-----	-----	-----	-----	----NKHA.
Rat	-----	-----	-----	-----	-HWM.HQQA.
					364
Arab.	QRYVLLCSKI	-PDGGFRDKP	RKPRDFYHTC	YCLSGLSVAQ	HAWLKDE-DT
Pea	.Q.I....QE	-Q...L....	G.R..H..S.	.....LC.	YS.S.RP-.S
Yeast	RD.I.Y.CQE	KEQP.L....	GAHS.....N	...L..A..E	SSYSCTPN.S
Rat	.E.I.M.CQC	..A..LL...	G.S.....	.....I..	-----HFGSG
					404
Arab.	PPLTRDIMGG	YSNLLEPVQL	LHNIVMDQYN	EAIEFFFKAA	-----
Pea	...PKVV..P	.....IHP	.F.V.L.R.R	..H...SQL-	-----
Yeast	.HNIKTPDR	LIGSSKLTDV	NPVYGLPIE.	VRKIIHYFKS	NLSSPS----
Rat	AM.HDVV..V	PE.V.Q.THP	VY..GP.KVI	Q.TTH.LQKP	VPGFEECEDA
Arab.	-----	-----	-----	-----	-----
Pea	-----	-----	-----	-----	-----
Yeast	-----	-----	-----	-----	-----
Rat	VTSDPATD--	-----	-----	-----	-----

FIGURE 4

FIG.5





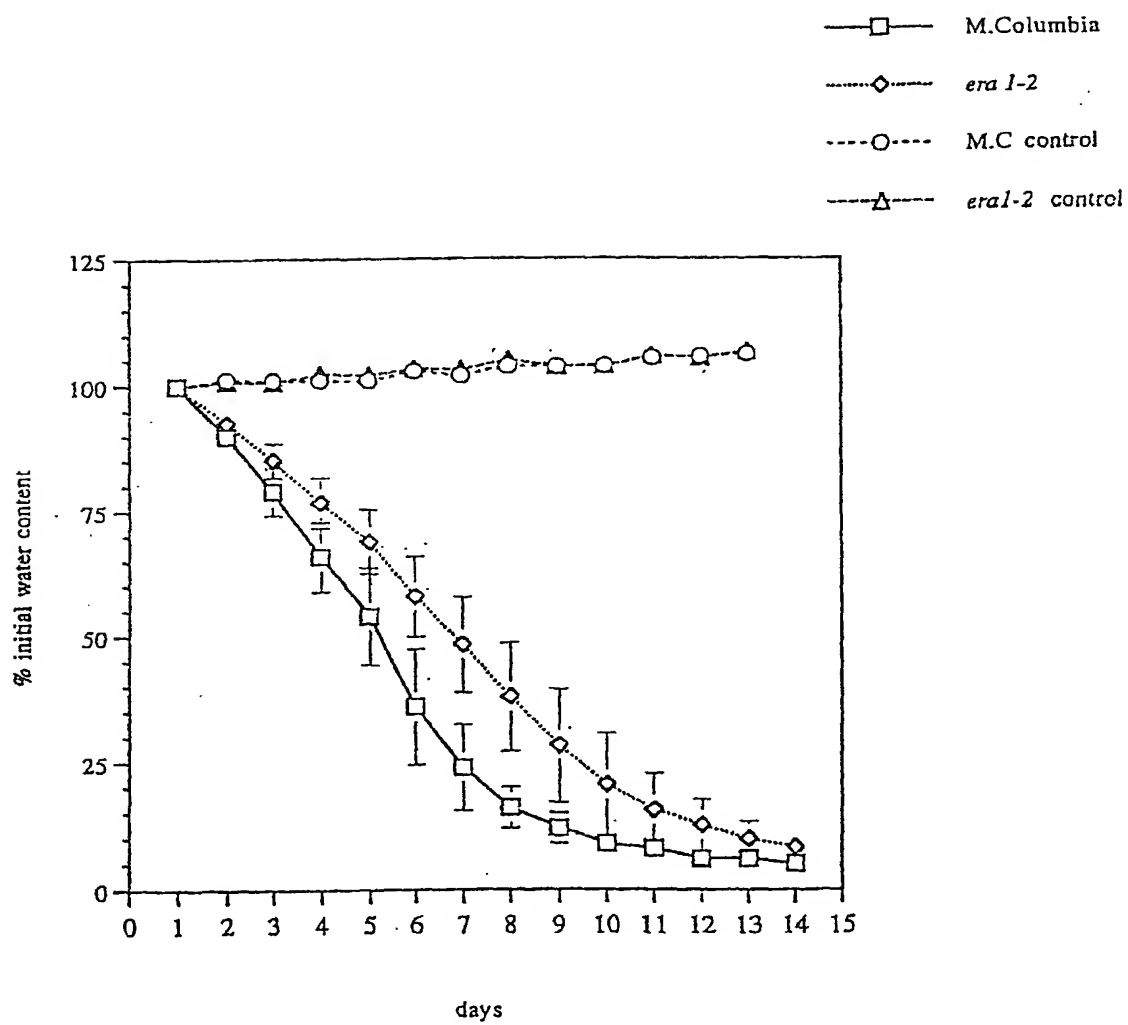


FIGURE 6

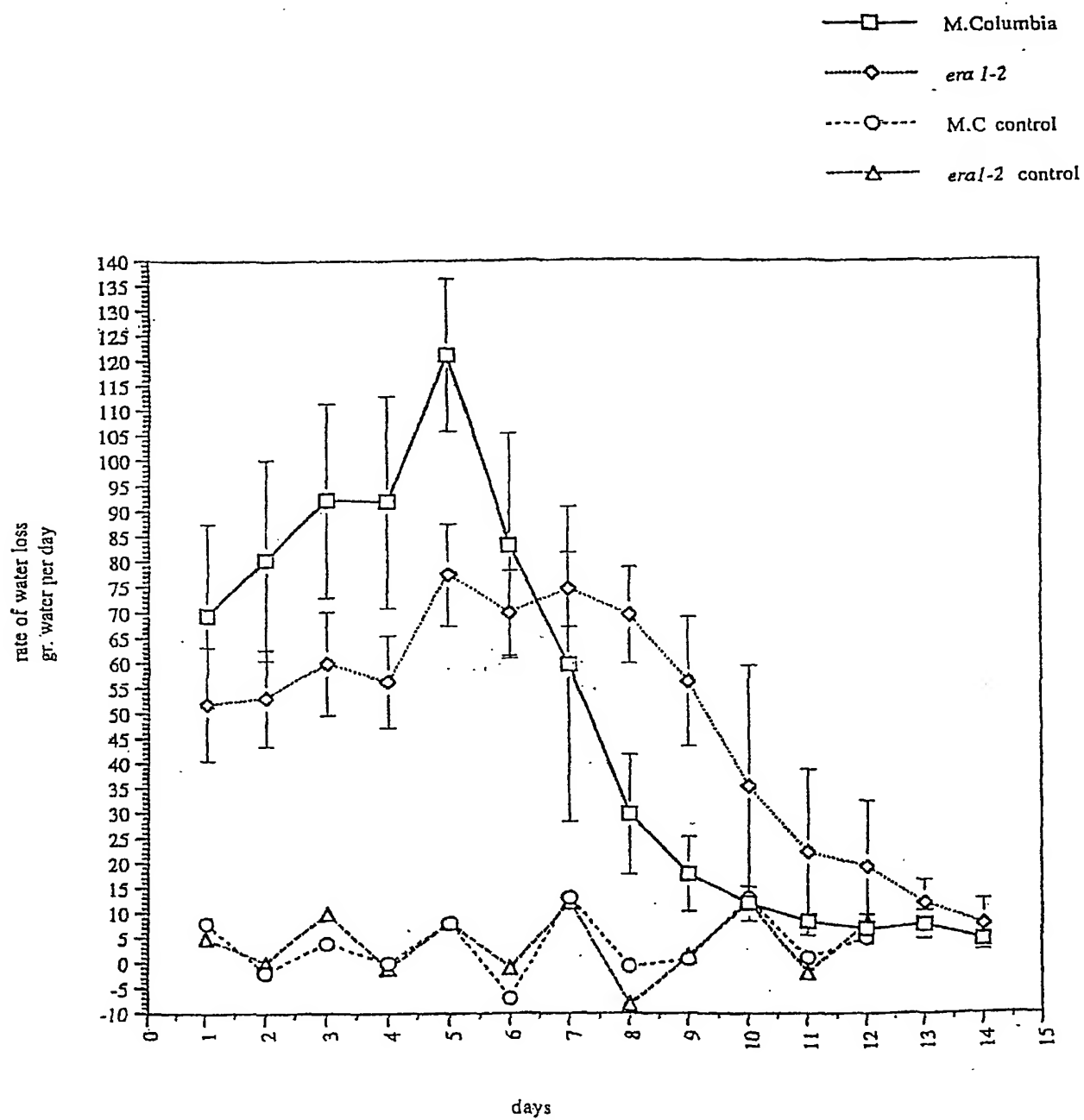


FIGURE 7

FIG.8A  
Day 0

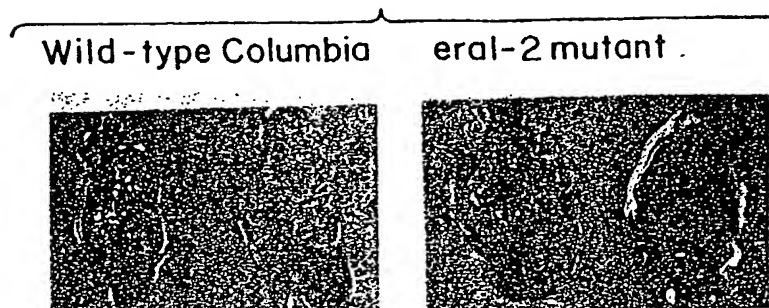


FIG.8B  
Day 3

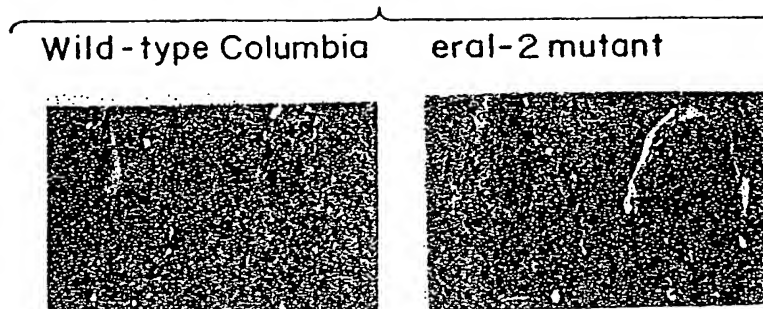


FIG.8C  
Day 6

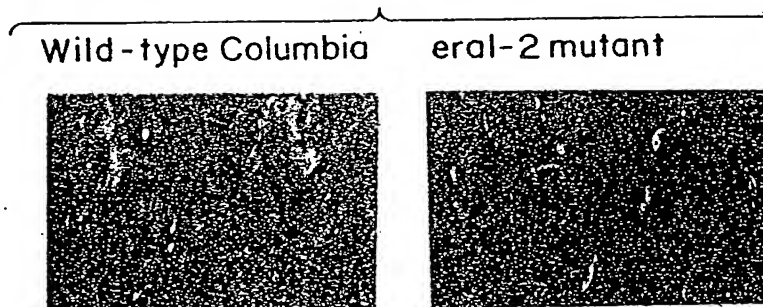


FIG.8D  
Day 9

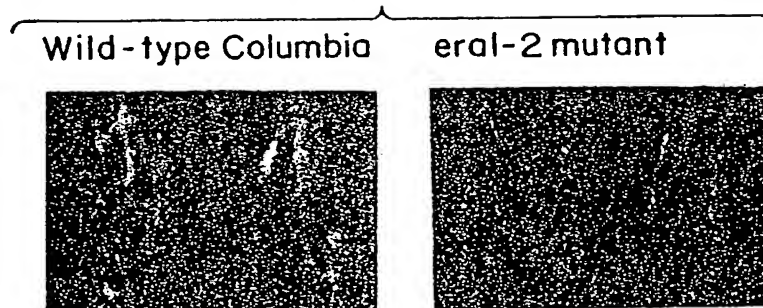
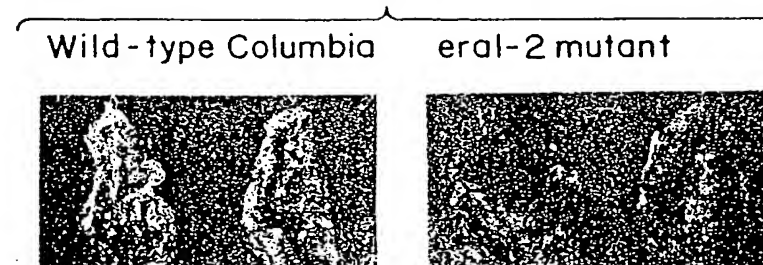
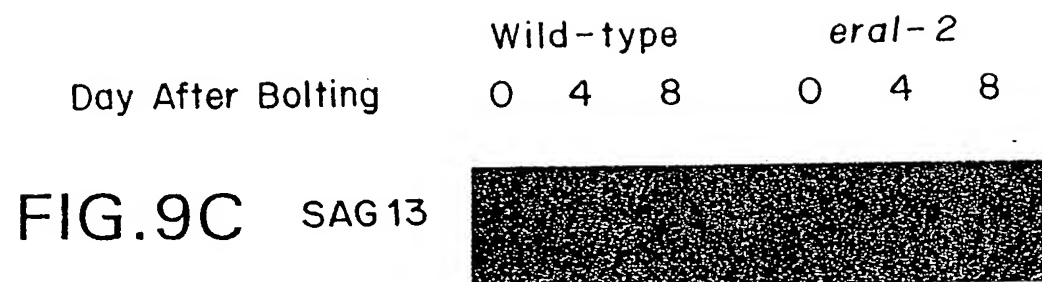
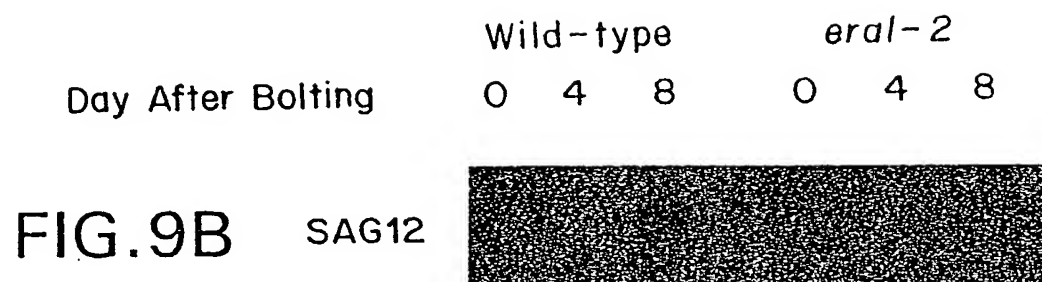
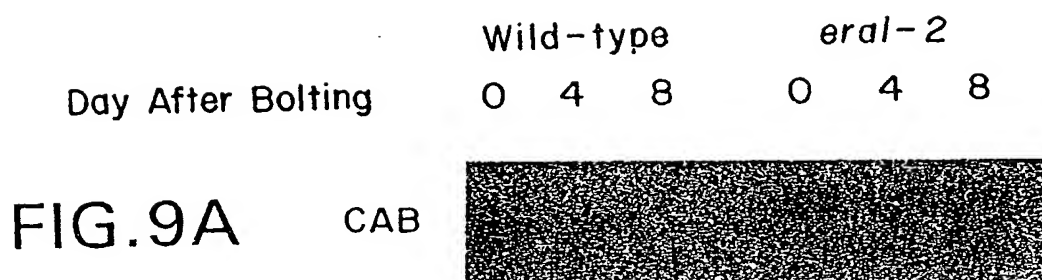


FIG.8E  
Day 12





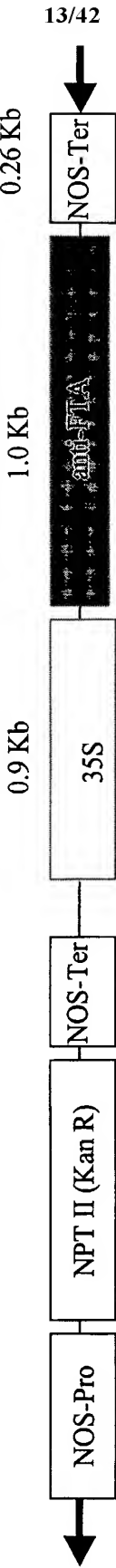


Figure 10

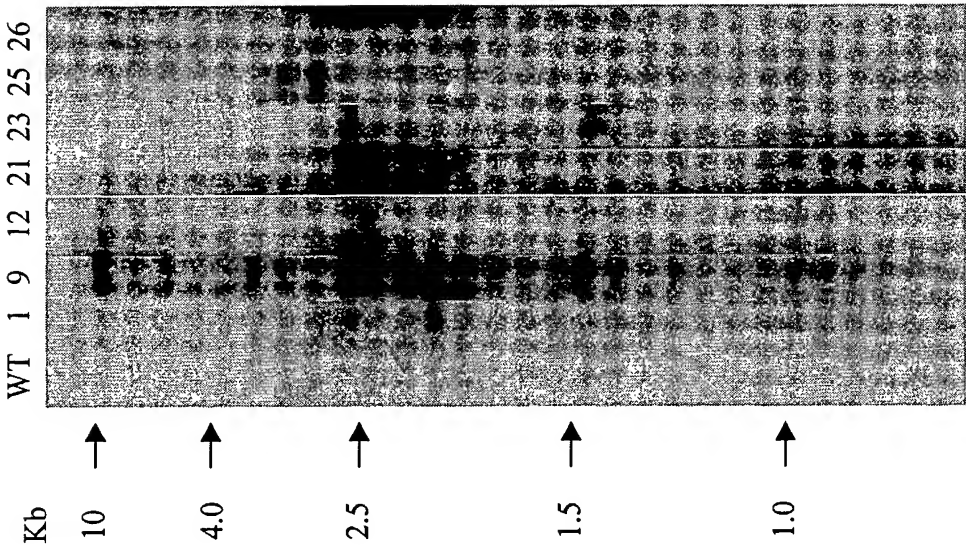
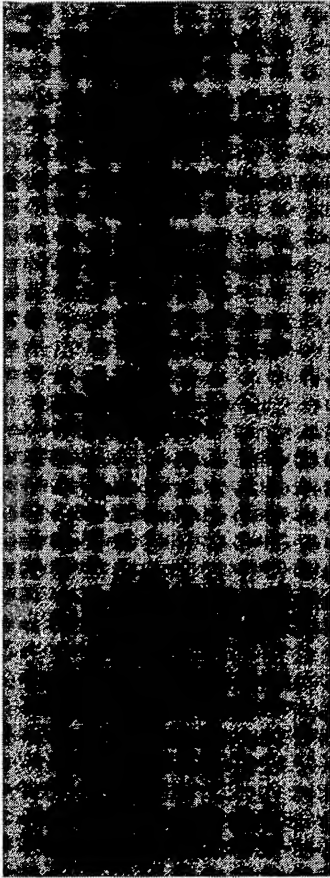
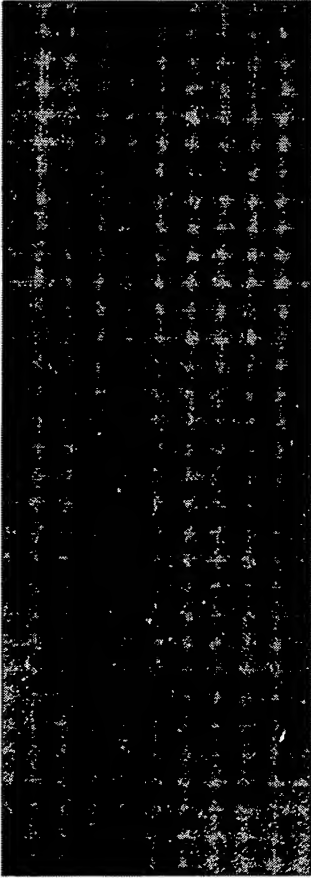


Figure 11

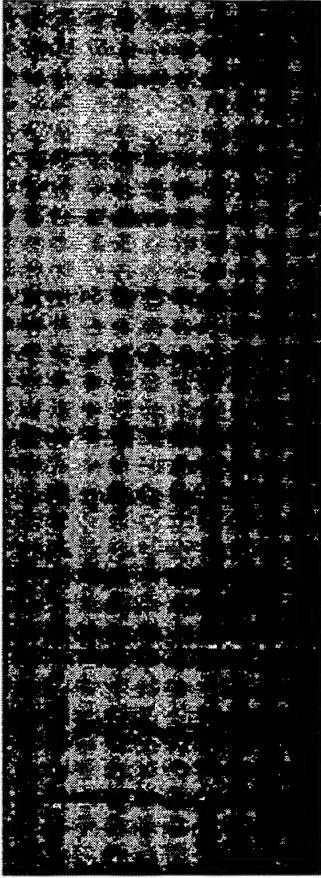
25 25 23 23 WT WT 12 12 21 21 26 26



A

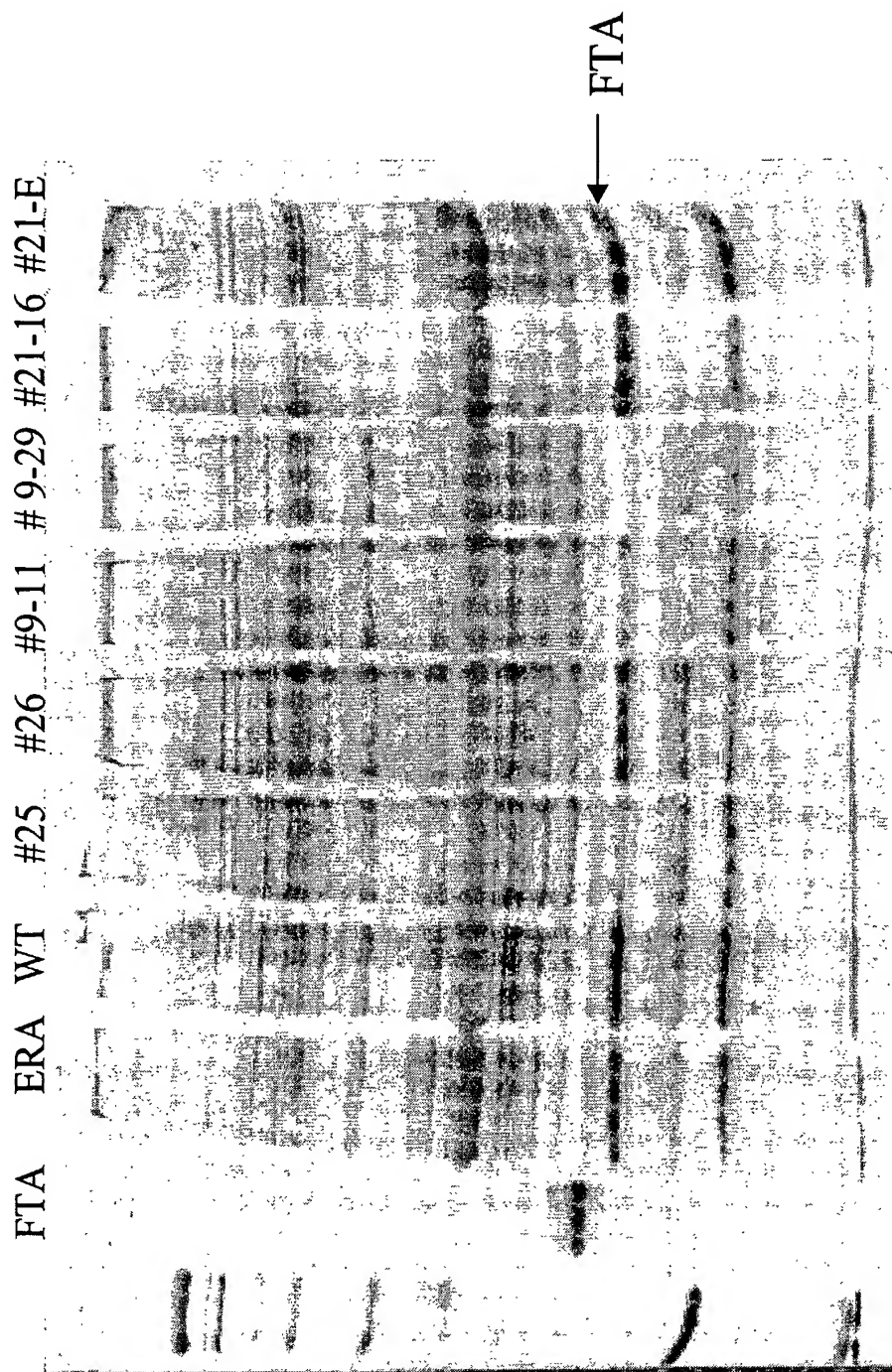


B



C

Figure 12

**Figure 13**



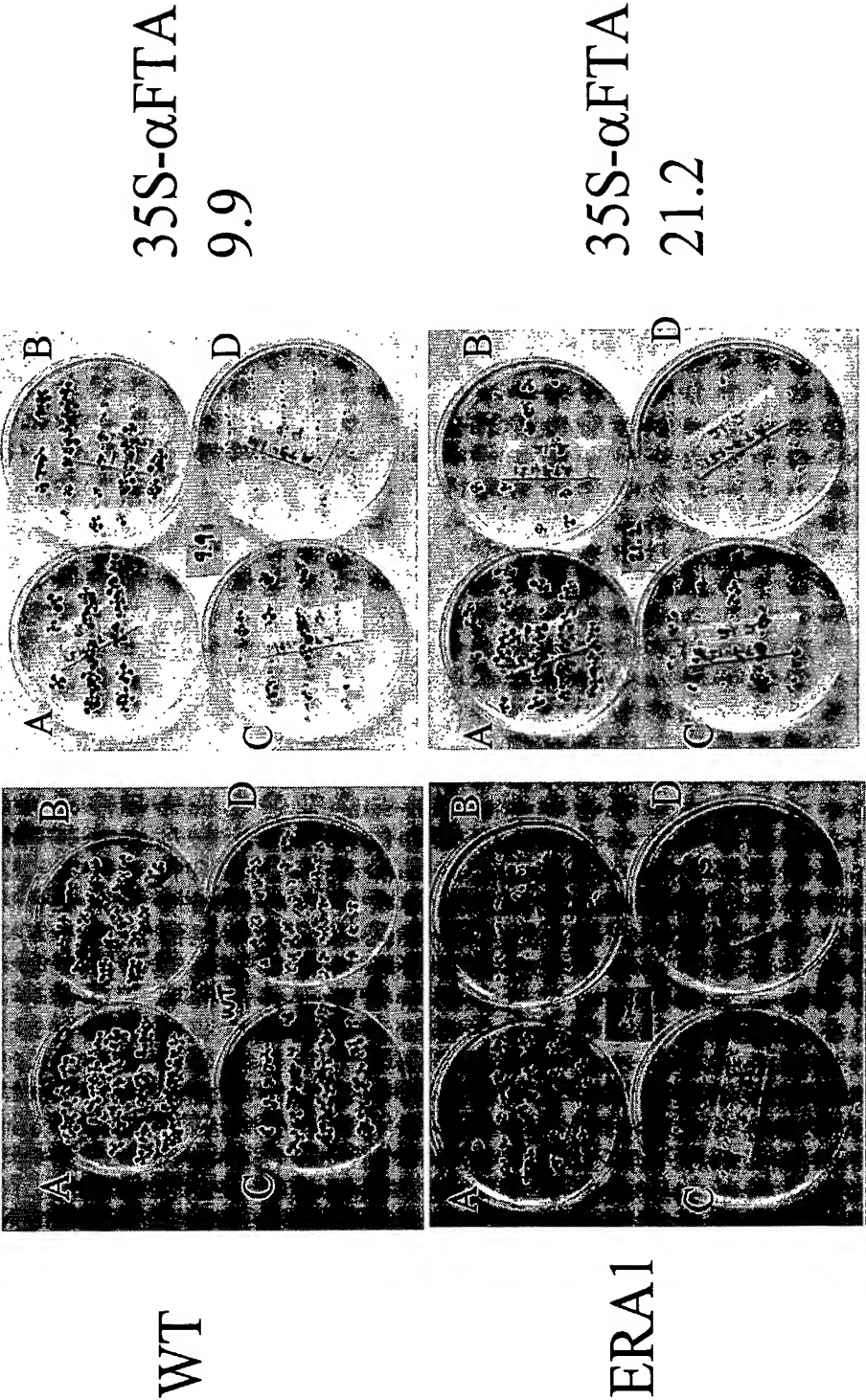


Figure 14

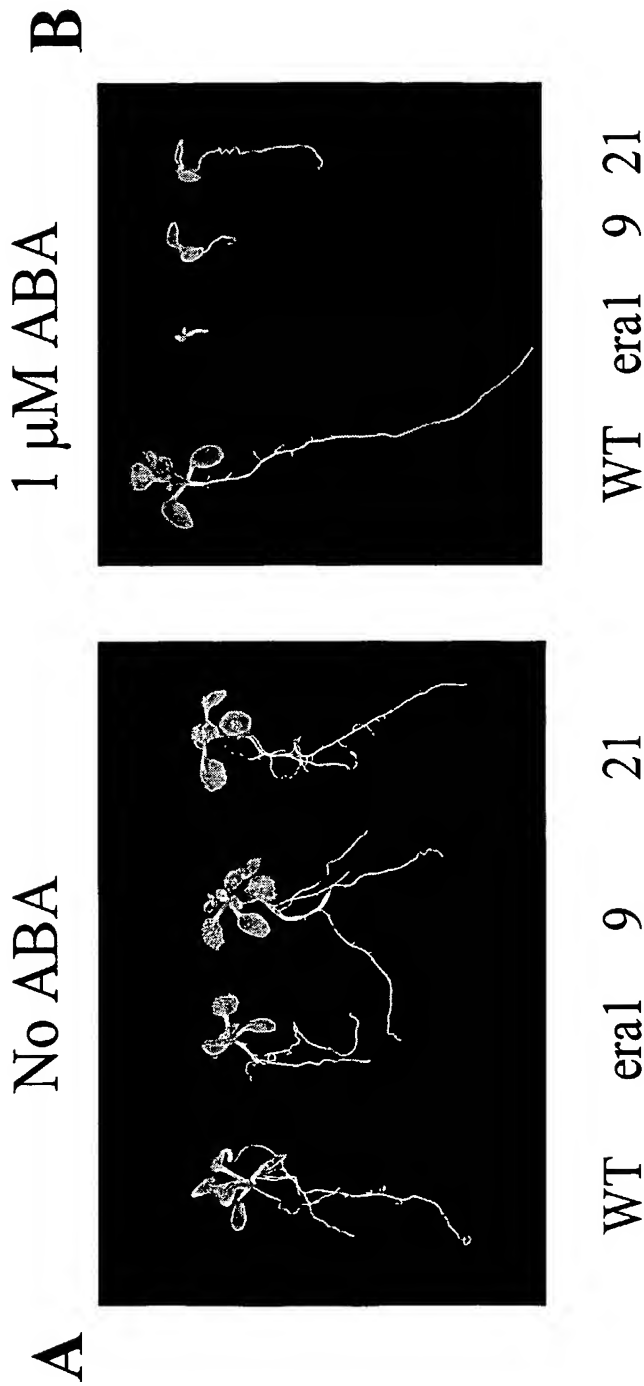


Figure 15

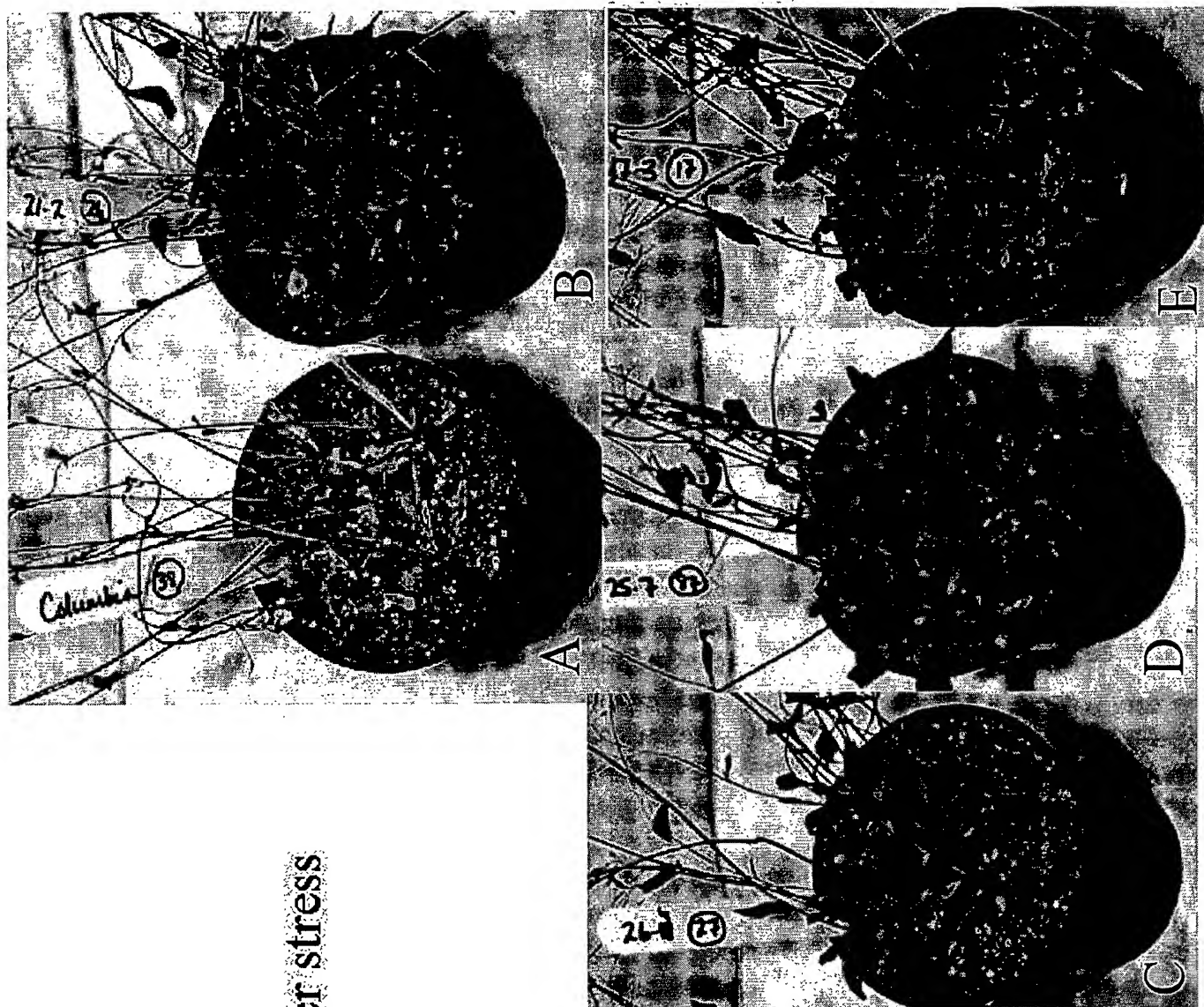


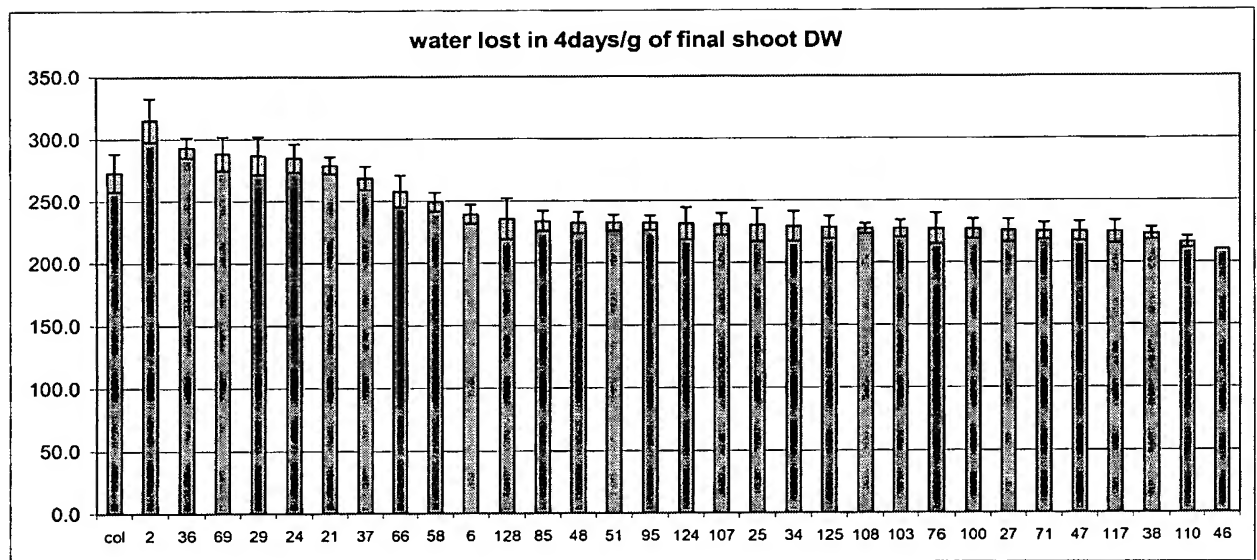
Figure 16.  
Day 8 of water stress

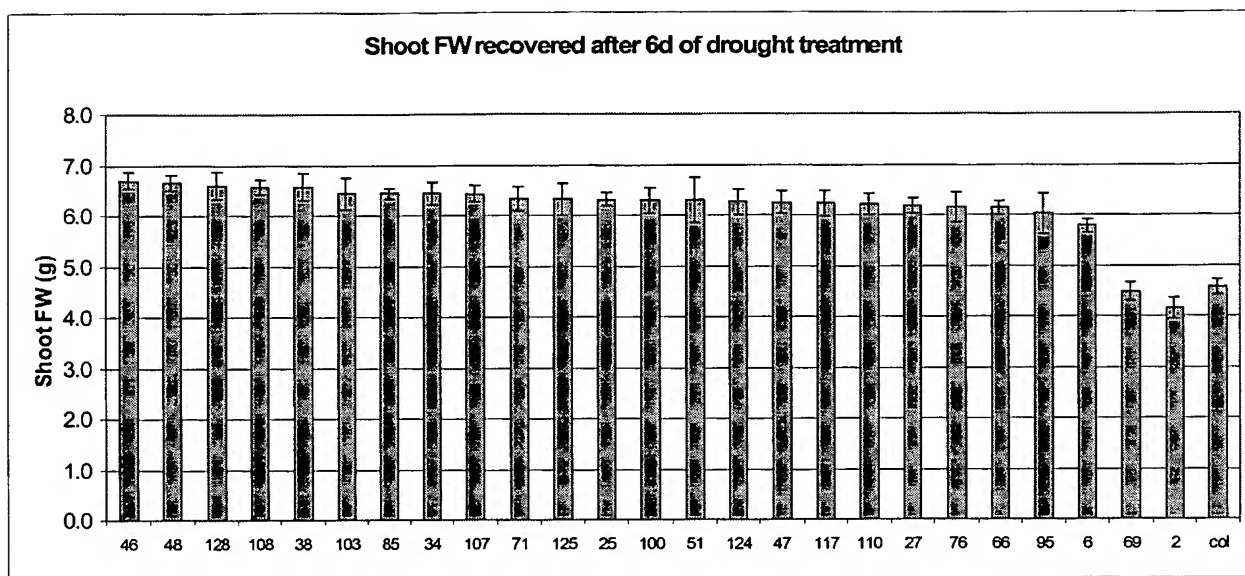
DNA	Brassica napus	Arabidopsis thaliana	PPI Glycine max	Zea mays	Rice	Soy 1	Soy 2	Triticum	Tomato	Pea
Brassica napus	X									
Arabidopsis thaliana	89	X								
PPI Glycine max	61	55	X							
Zea mays	57	45	52	X						
Rice	55	46	54	63	X					
Soy 1	61	50	98	43	47	X				
Soy 2	61	50	99	41	46	99	X			
Triticum	58	45	52	56	66	43	41	X		
Tomato	65	53	63	44	51	52	49	41	X	
Pea	66	55	78	46	50	70	69	44	49	X
<b>PROTEIN</b>	<b>Brassica napus</b>	<b>Arabidopsis thaliana</b>	<b>PPI Glycine max</b>	<b>Pea</b>	<b>Tomato</b>	<b>Rice</b>	<b>Zea mays</b>	<b>Soy 1</b>	<b>Soy 2</b>	<b>Triticum</b>
Brassica napus	X									
Arabidopsis thaliana	89	X								
PPI Glycine max	65	63	X							
Pea	61	61	77	X						
Tomato	60	59	57	58	X					
Rice	64	63	56	58	58	X				
Zea mays	61	56	58	57	56	75	X			
Soy 1	66	64	98	77	58	57	58	X		
Soy 2	66	64	98	78	58	57	58	99	X	
Triticum	61	60	57	59	60	80	73	58	58	X

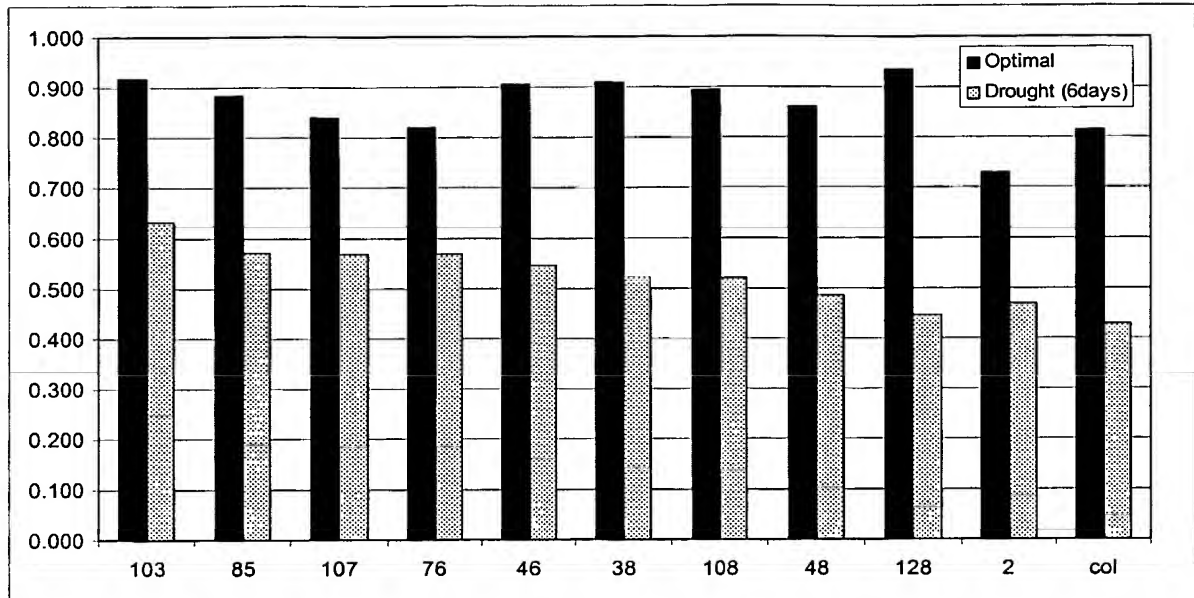
Figure 17

DNA	Brassica napus	Arabidopsis thaliana	Wiggum	PPI Glycine max	Glycine max	PPI Zea maize	Zea maize	Pea	Tomato	Tobacco
Brassica napus	X									
Arabidopsis thaliana	88	X								
Wiggum	88	99	X							
PPI Glycine max	60	64	65	X						
Glycine max	60	64	65	99	X					
PPI Zea maize	38	54	59	63	63	X				
Zea maize	54	54	59	62	62	99	X			
Pea	65	57	45	78	77	56	56	X		
Tomato	68	62	52	70	70	64	64	51	X	
Tobacco	68	64	60	71	71	65	65	55	83	X
<b>PROTEIN</b>	Brassica napus	Arabidopsis thaliana	Wiggum	PPI Glycine max	Glycine max	PPI Zea maize	Zea maize	Pea	Tomato	Tobacco
Brassica napus	X									
Arabidopsis thaliana	84	X								
Wiggum	84	99	X							
PPI Glycine max	54	58	59	X						
Glycine max	53	58	58	99	X					
PPI Zea maize	52	50	52	58	58	X				
Zea maize	51	50	52	58	58	99	X			
Pea	58	56	57	78	78	56	56	X		
Tomato	60	62	55	63	63	58	58	62	X	
Tobacco	62	63	59	64	63	58	58	64	83	X

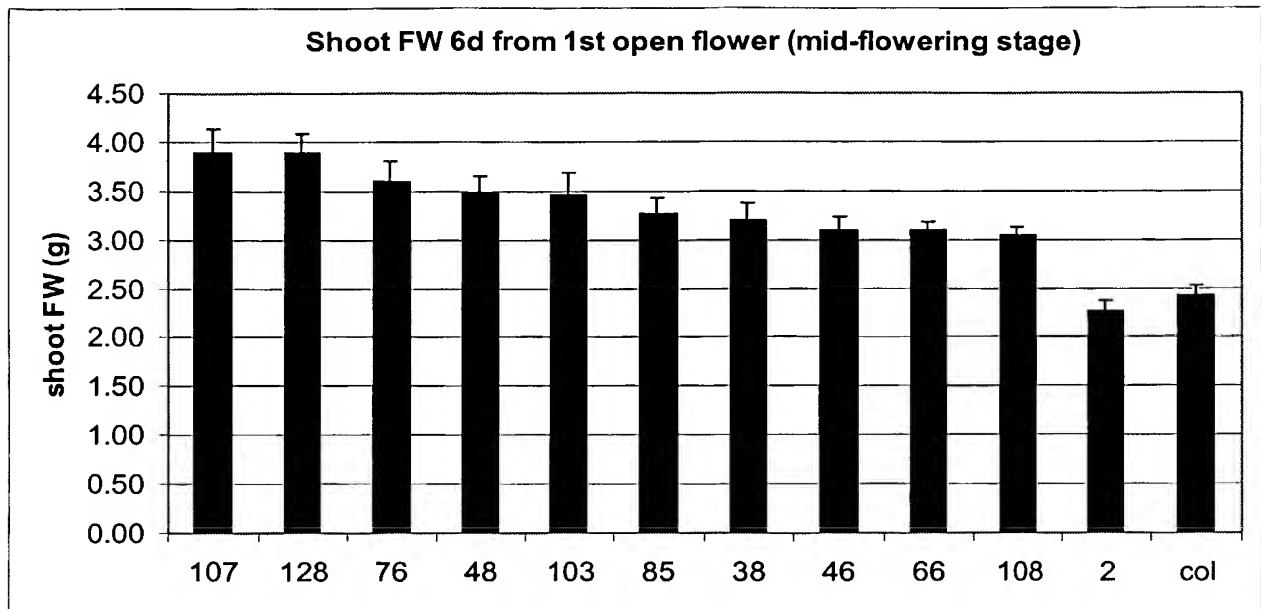
Figure 18

**Figure 19**

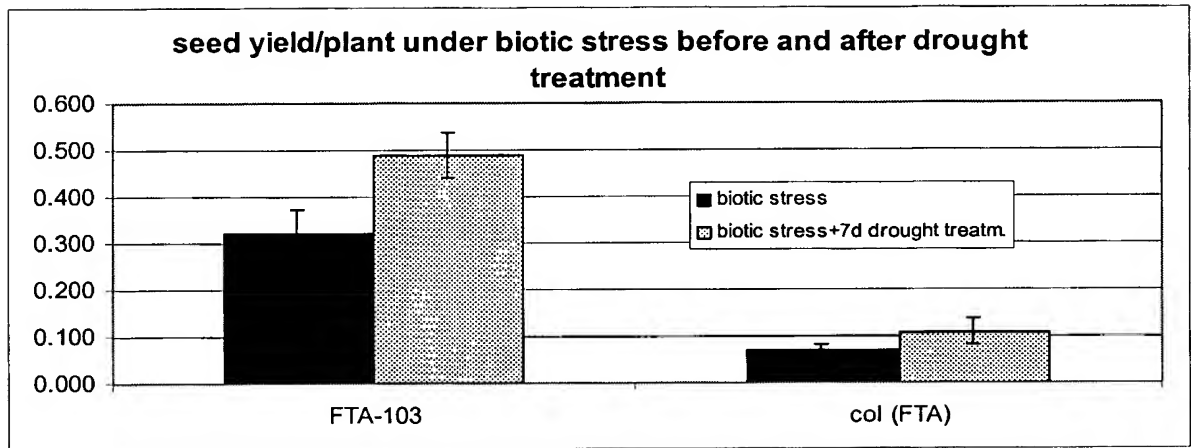
**Figure 20**

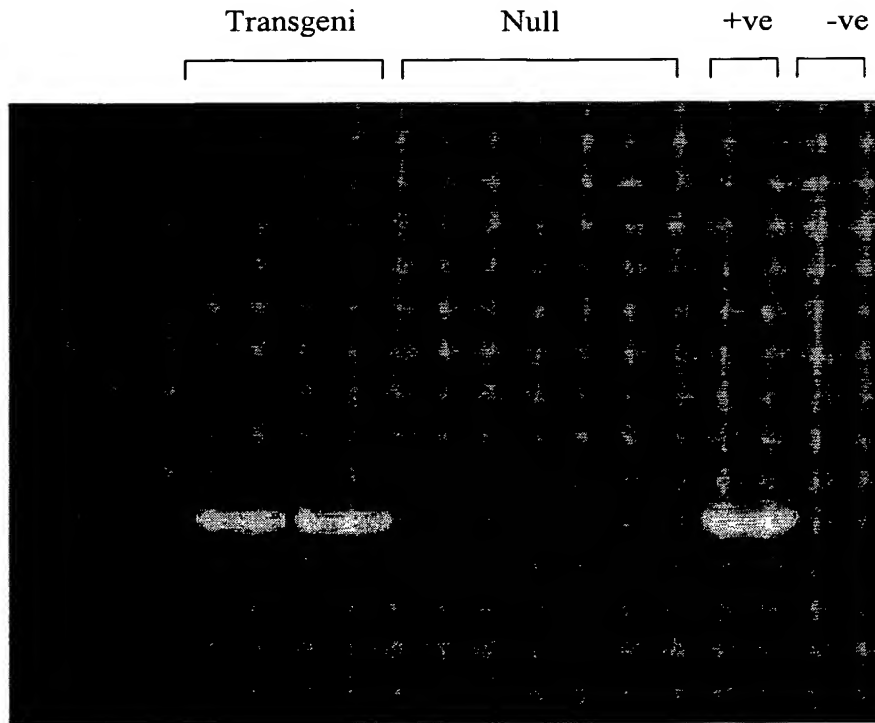
**Figure 21**





**Figure 22**

**Figure 23**

**Figure 24**

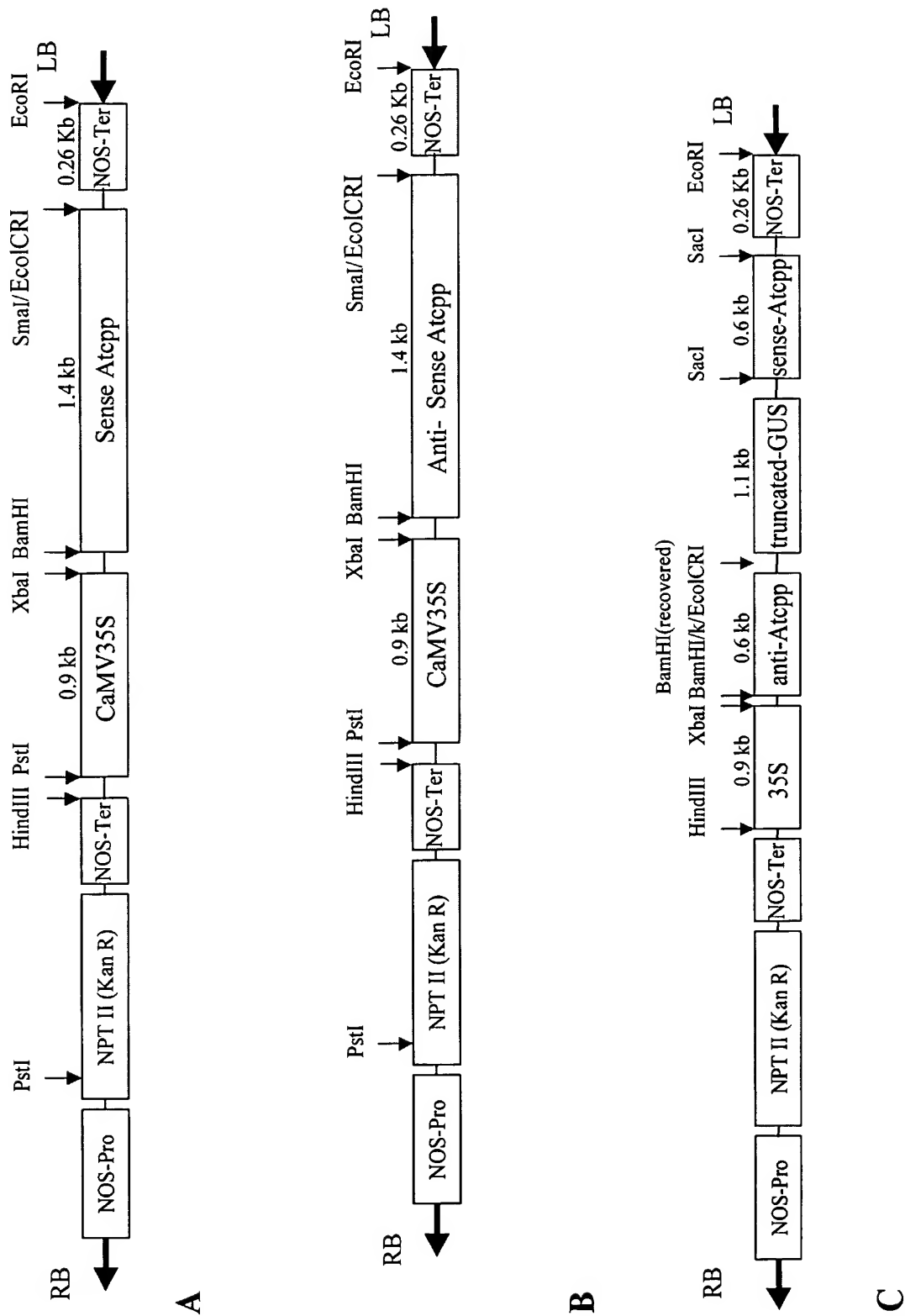


Figure 25.

**A**

Nucleic Acid	PPI-AtCPP	PPI-BnCPP	PPI-SoyCPP	BASF-AT1	BASF-AT2	BASF-Corn	BASF-Soy	AFC1	AT4g01320	AF007269
PPI-AtCPP	X									
PPI-BnCPP	92	X								
PPI-SoyCPP	76	77	X							
BASF-AT1	98	93	76	X						
BASF-AT2	99	93	76	99	X					
BASF-Corn	57	57	57	57	57	X				
BASF-Soy	72	72	93	72	72	52	X			
AFC1	99	93	77	99	99	57	72	X		
AT4g01320	99	92	70	99	99	50	64	99	X	
AF007269	97	91	10	97	97	13	8	97	97	X

**B**

Amino Acid	PPI-AtCPP	PPI-BnCPP	PPI-SoyCPP	BASF-AT1	BASF-AT2	BASF-Corn	BASF-Soy	AFC1	AT4g01320	AF007269
PPI-AtCPP	X									
PPI-BnCPP	94	X								
PPI-SoyCPP	83	83	X							
BASF-AT1	98	95	83	X						
BASF-AT2	99	95	83	99	X					
BASF-Corn	82	82	79	82	82	X				
BASF-Soy	83	83	99	83	83	73	X			
AFC1	98	95	83	99	99	82	83	X		
AT4g01320	95	93	82	96	96	72	76	96	X	
AF007269	98	94	82	98	99	82	82	98	100	X

Figure 26

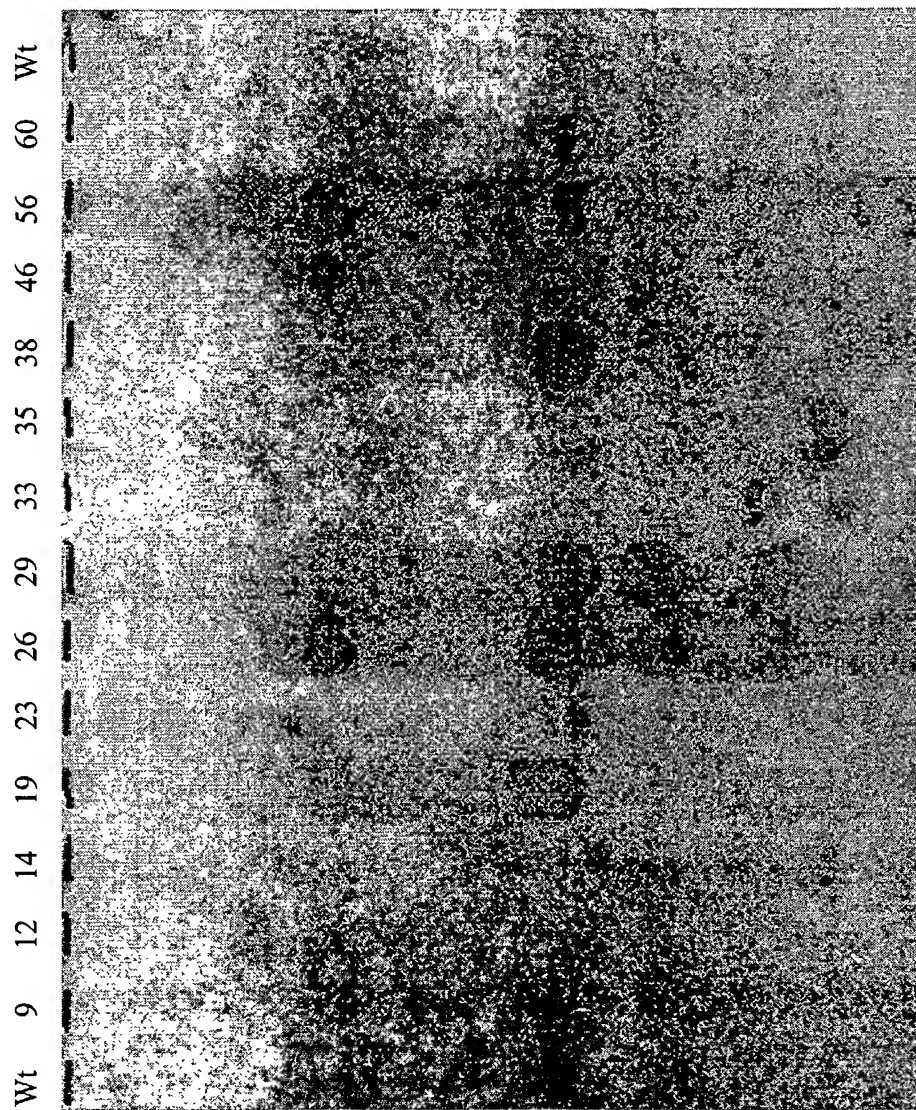


Figure 27.

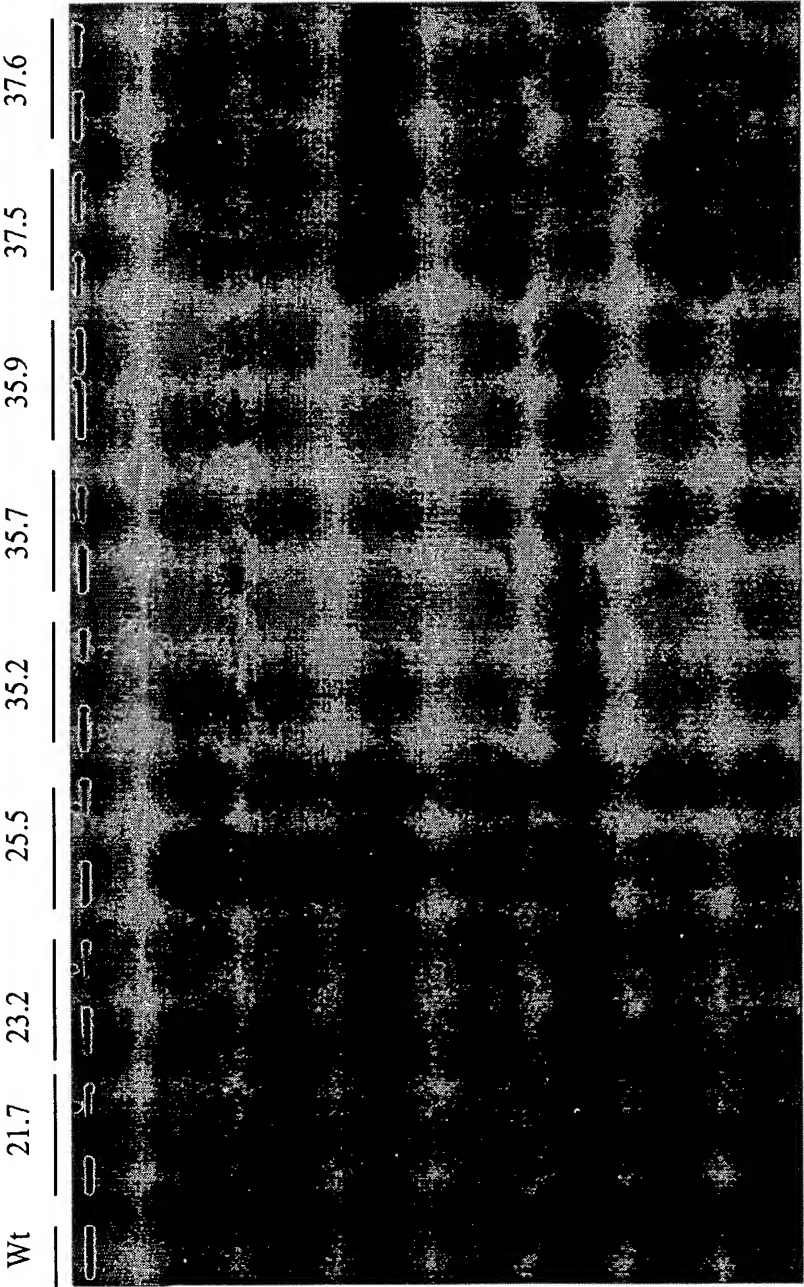


Figure 28.

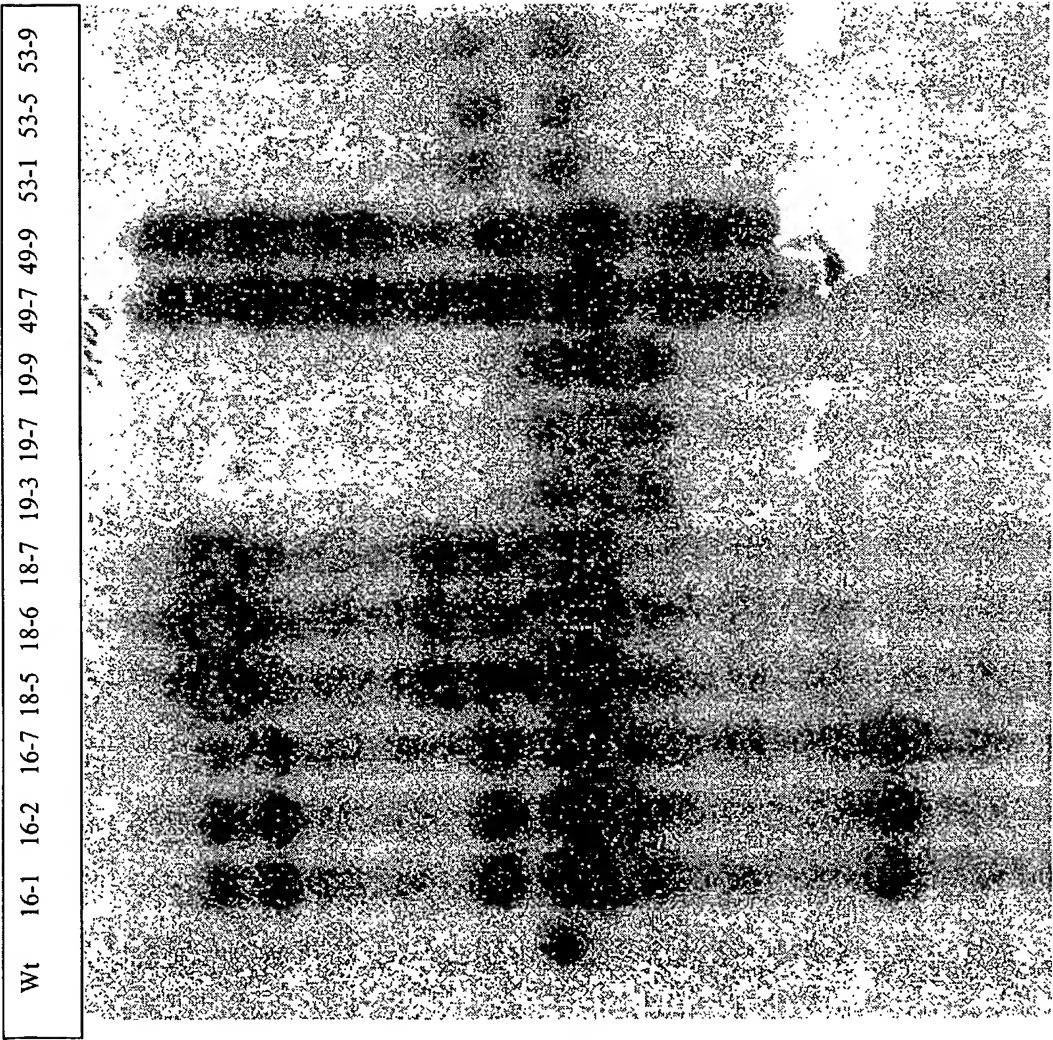


Figure 29



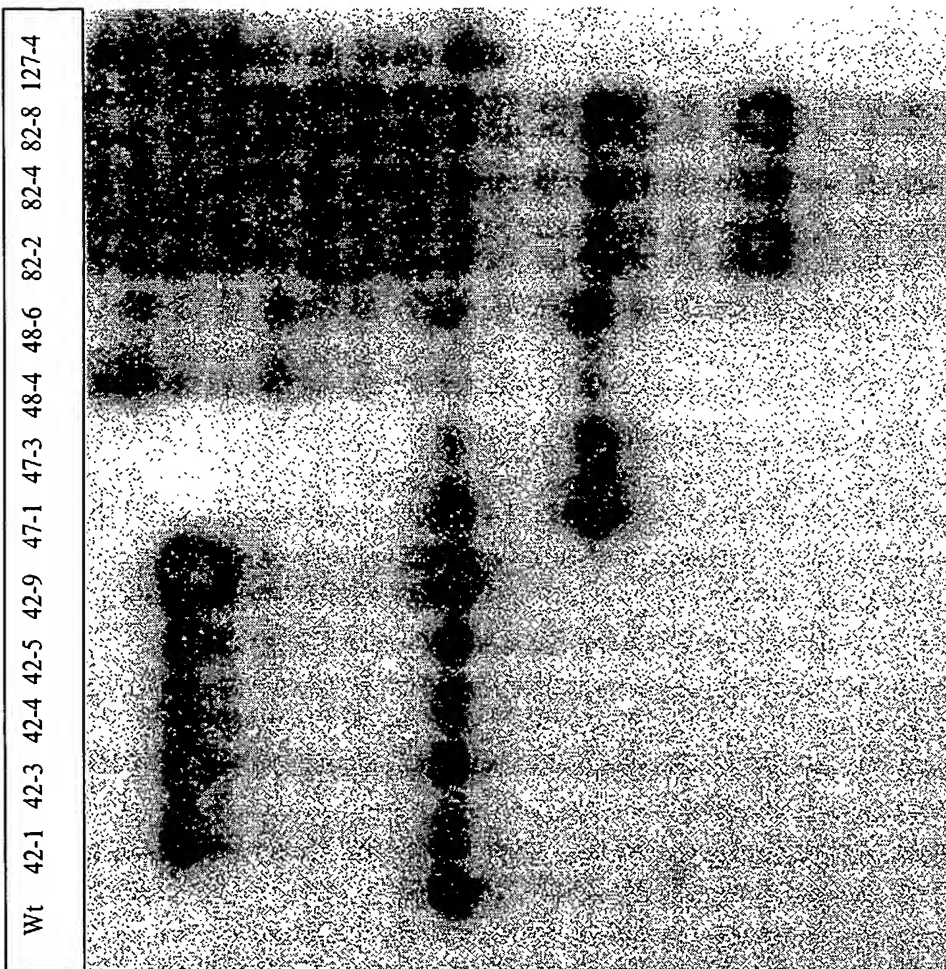


Figure 30

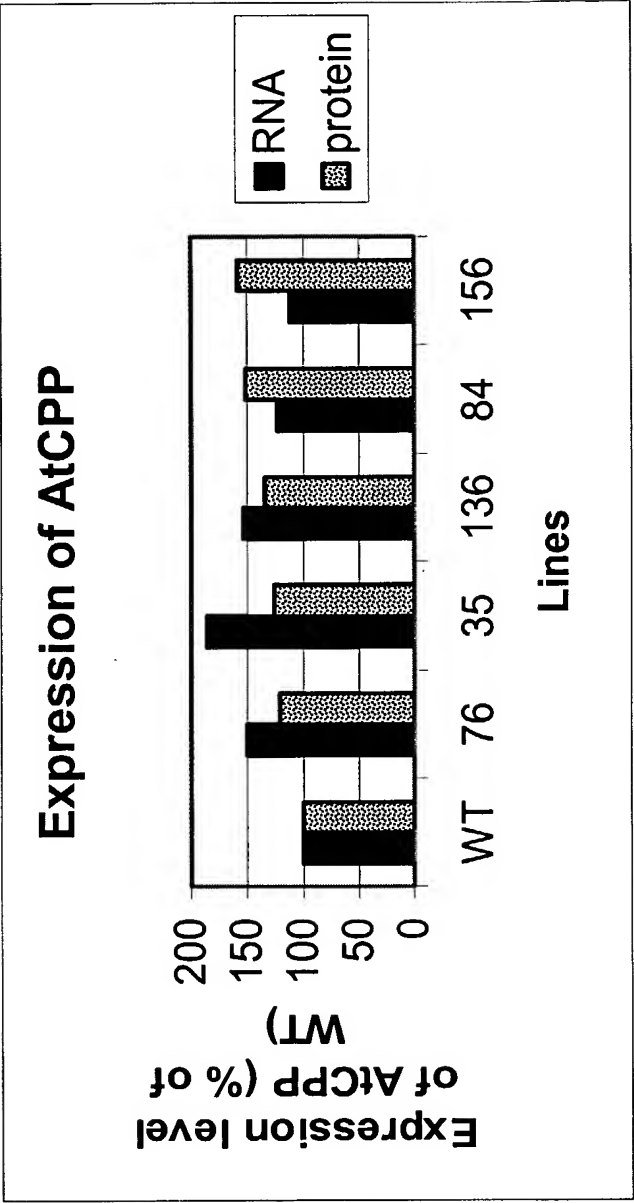


Figure 31

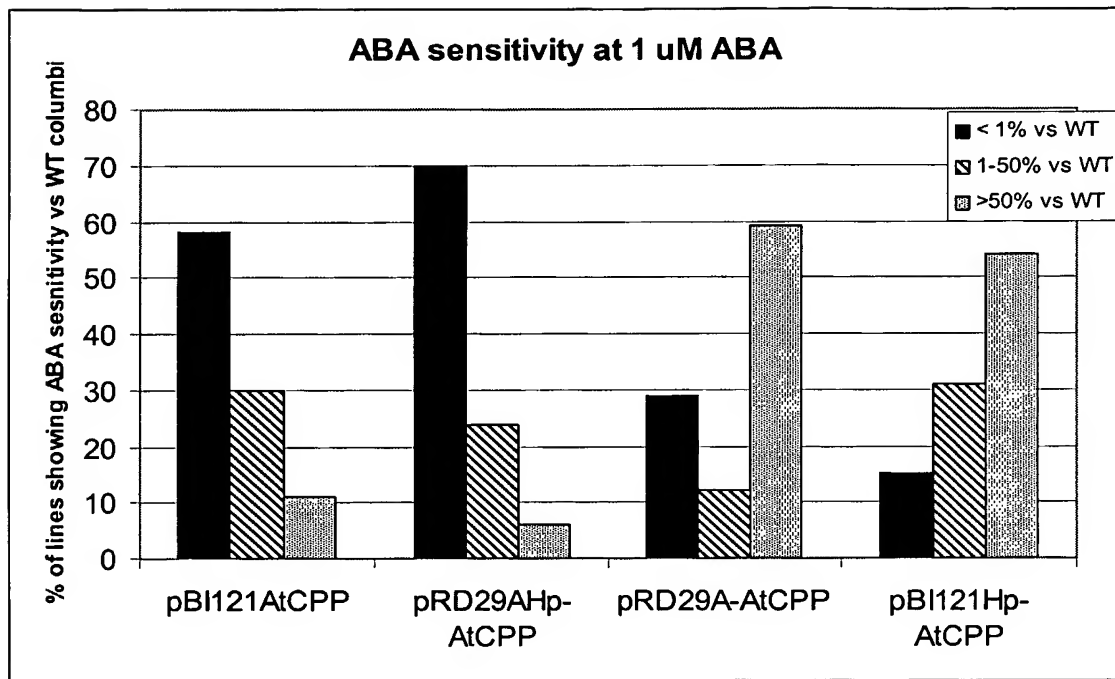


Figure 32

2 weeks old seedling on different [ABA]

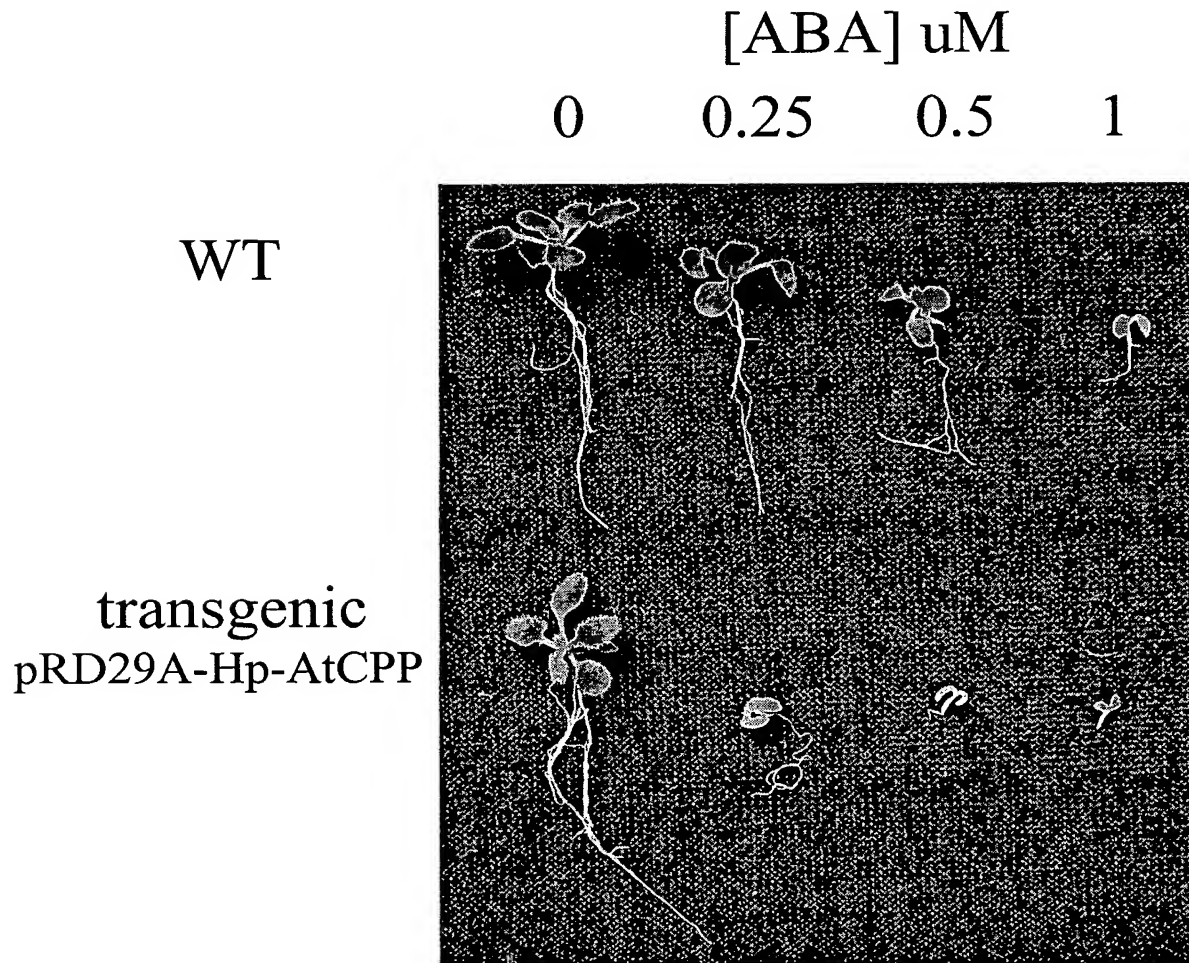


Figure 33

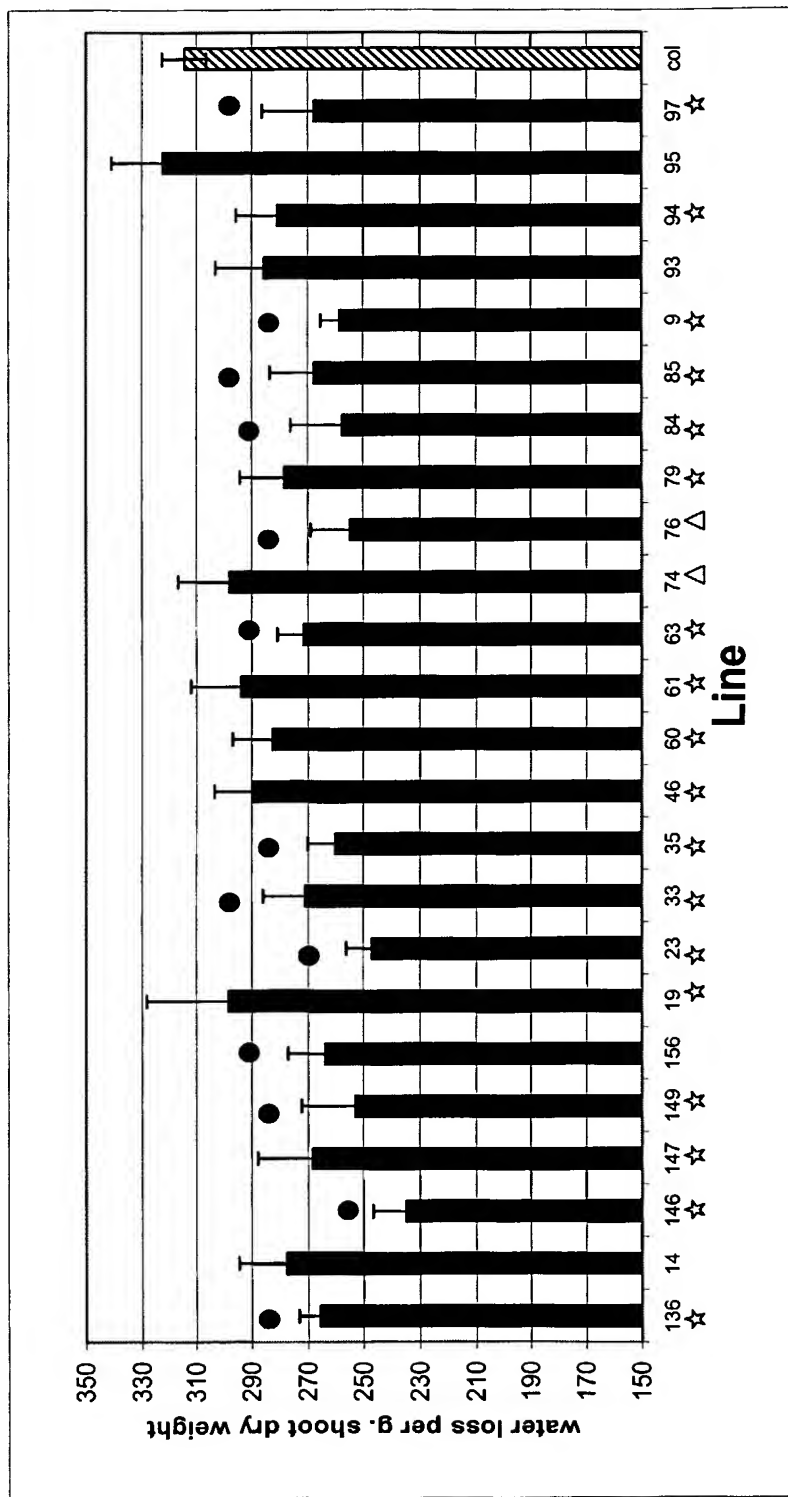


Figure 34.

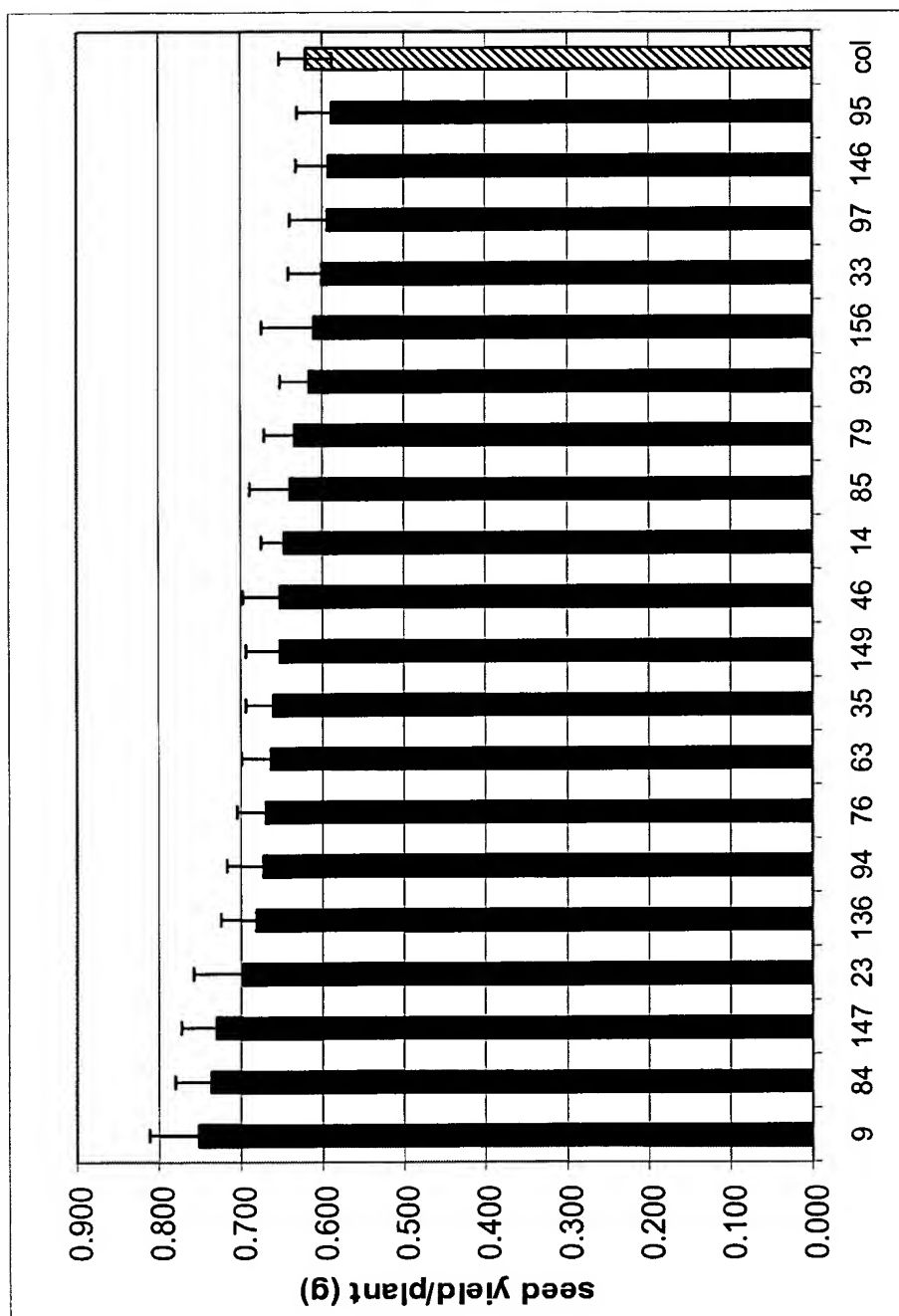


Figure 35

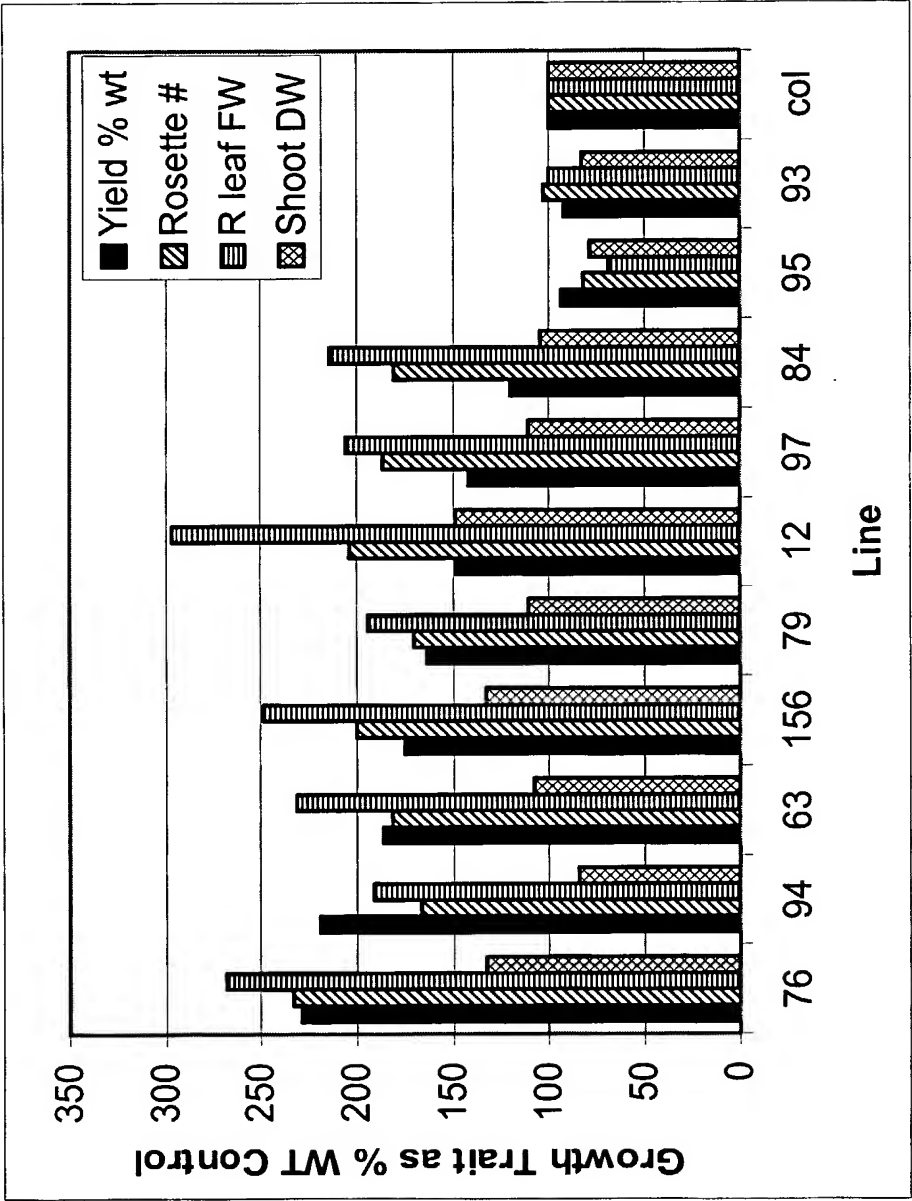


Figure 36

35S-AtCPP 12 days old seedlings - toluidin blue

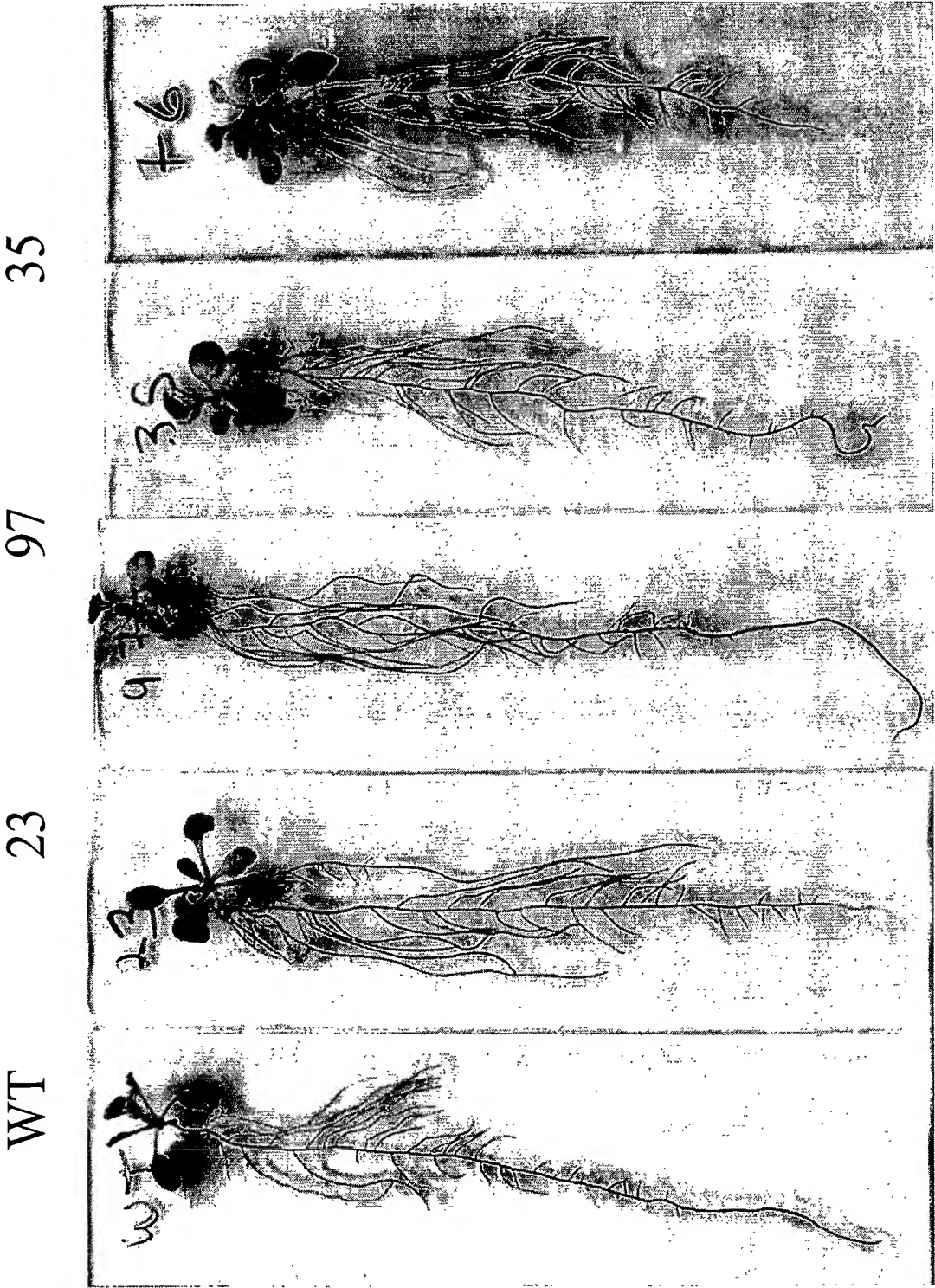


Figure 37



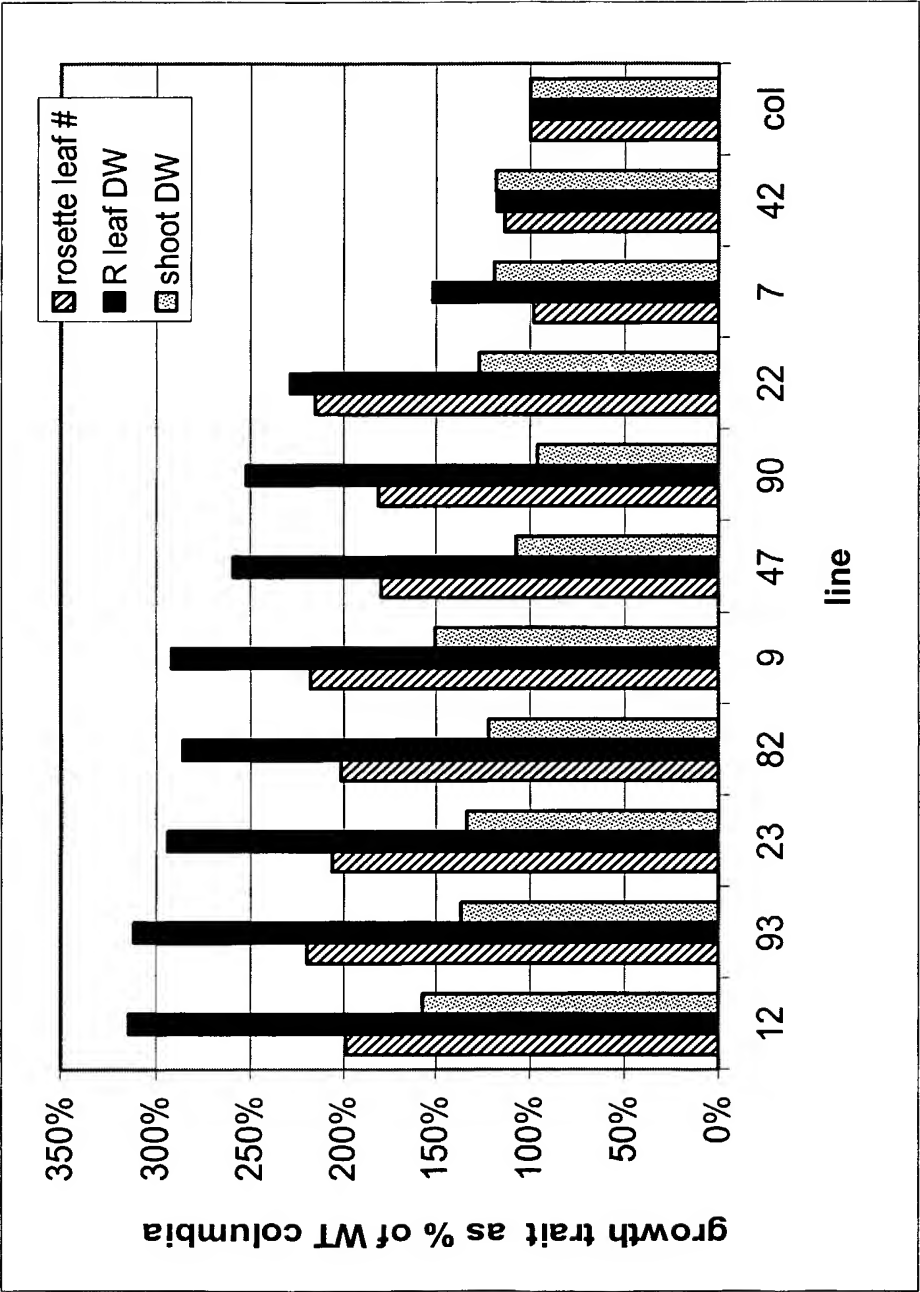


Figure 38

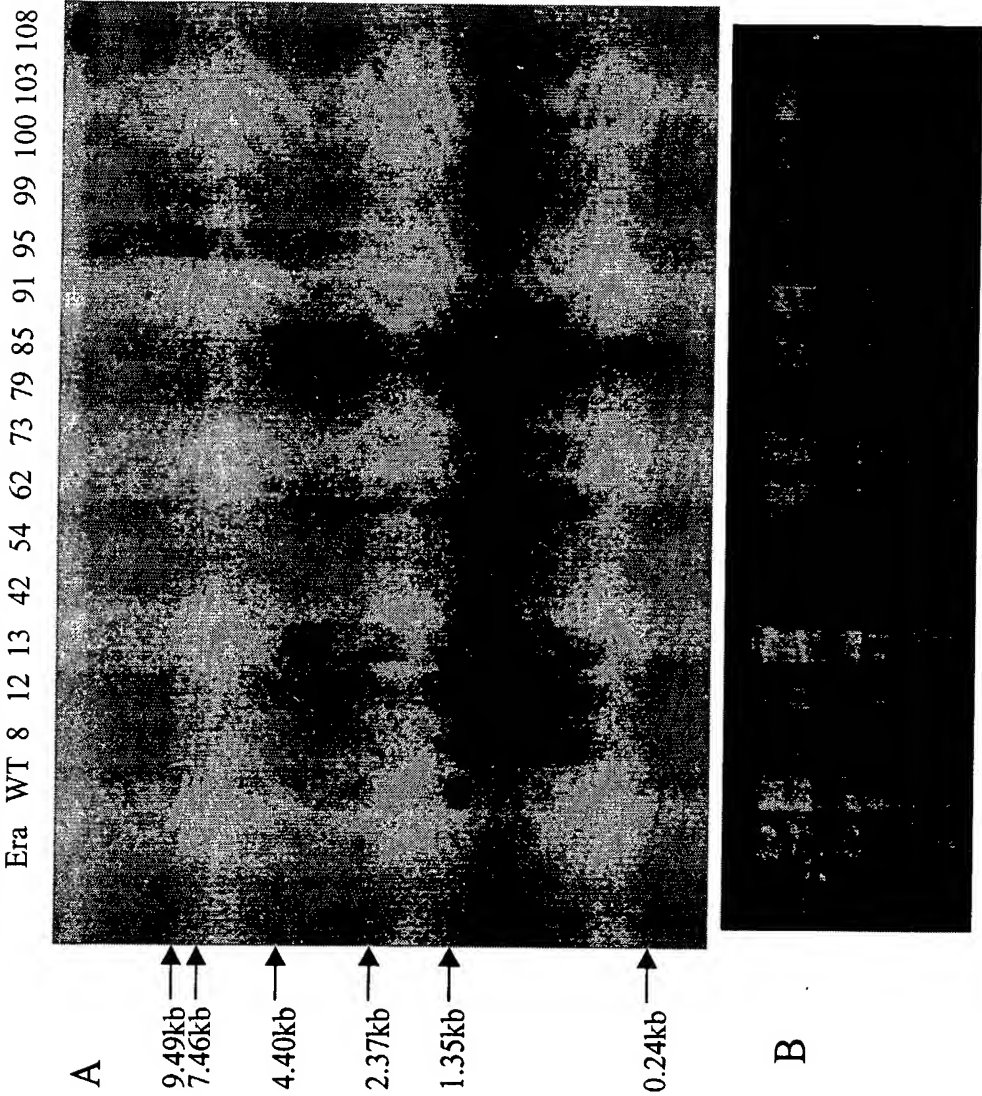


Figure 39 . Northern blot of  $\Delta N90AtFTB$  arabidopsis plants

A. Northern blot probed with  $\Delta N90AtFTB$  DNA probe

B. Ethidium bromide stain of agarose gel showing RNA loading per lane